ATP BIOLUMINESCENCE A NEW LIGHT FOR MONITORING MICROORGANISMS ON DRINKING WATER AND WASTEWATER

Presenter:

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The Water Treatment Plant, City of Pittsburg.

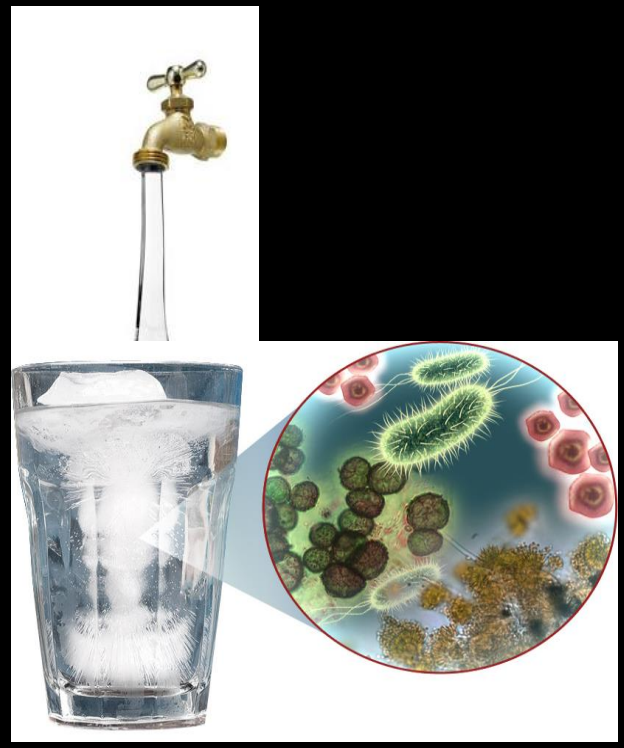
baywork

bay area water/wastewater workforce development

SEVERAL INSTRUMENTS GIVE WATER MANAGERS THE ABILITY TO QUICKLY AND EASILY ASSESS SEVERAL WATER QUALITY PARAMETERS, SUCH AS:

- Temperature
- pH
- Alkalinity
- Turbidity
- Color
- TDS
- Chlorine residual

- What about biological activity?




At least we won't have to do this for another 3 or 5 years!


Hillsdale Dr
Alta Vista Cir

NITRIFICATION ISSUES

NITRITE/NITRATE FORMATION

UNDER THE SAFE DRINKING WATER ACT (SDWA), PRIMARY MCLS HAVE BEEN ESTABLISHED FOR NITRITE-N, NITRATE-N, AND THE SUM OF NITRITE-N PLUS NITRATE-N.
THE MCLS IS:

1 MG/L FOR NITRITE-N

10 MG/L FOR NITRATE-N

AND

10 MG/L FOR NITRITE + NITRATE (AS N).

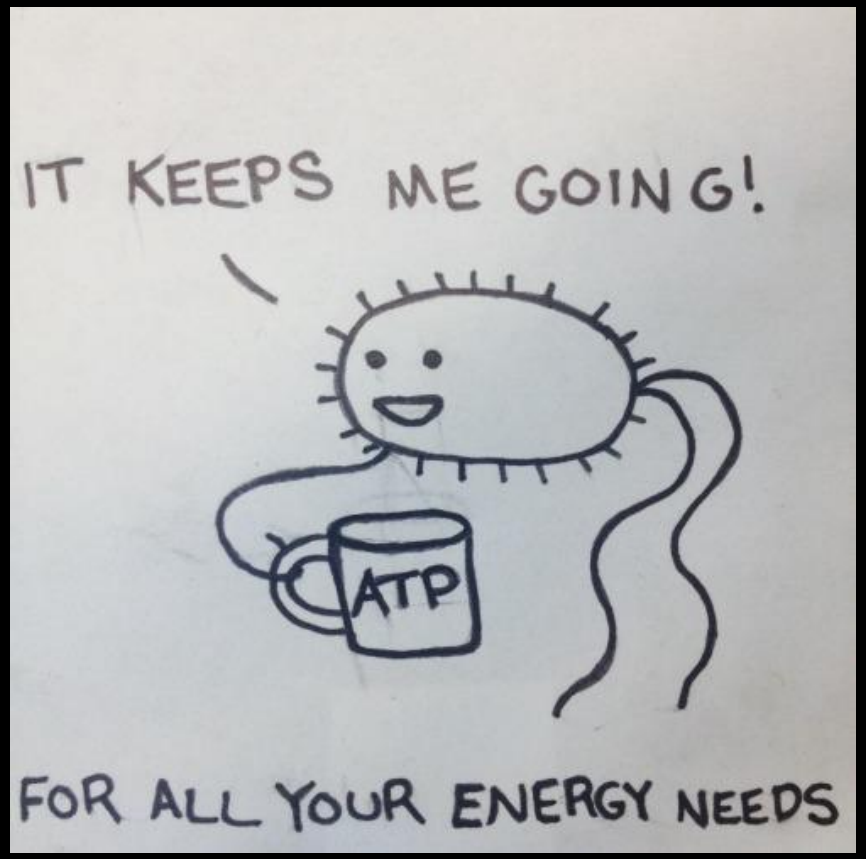
THE CURRENT NITRITE AND NITRATE STANDARDS ARE MEASURED AT THE POINT OF ENTRY TO THE DISTRIBUTION SYSTEM SO ANY SUBSEQUENT ELEVATED NITRITE/NITRATE LEVELS RESULTING FROM NITRIFICATION WITHIN THE DISTRIBUTION SYSTEM ARE NOT IDENTIFIED BY COMPLIANCE MONITORING.

CHEMICAL ISSUES AND BIOLOGICAL ISSUES

Chemical Issues	→	**Biological Issues**
• Disinfectant Depletion	→	HPC Increase
• Nitrite/Nitrate Formation Increase	→	Ammonia Oxidizing Bacteria (AOB) Nitrite Oxidizing Bacteria (NOB)

- Reduction in pH and Alkalinity
- DBP Formation due to Mitigation Techniques

ATP-BIOLUMINESCENCE
ATP - BIOLUMINESCENCE PRINCIPLE



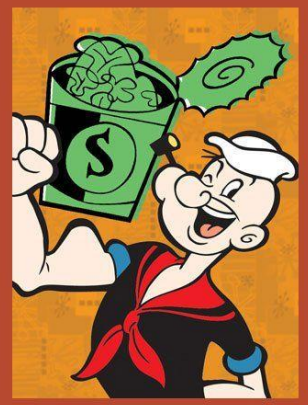
ATP Basics

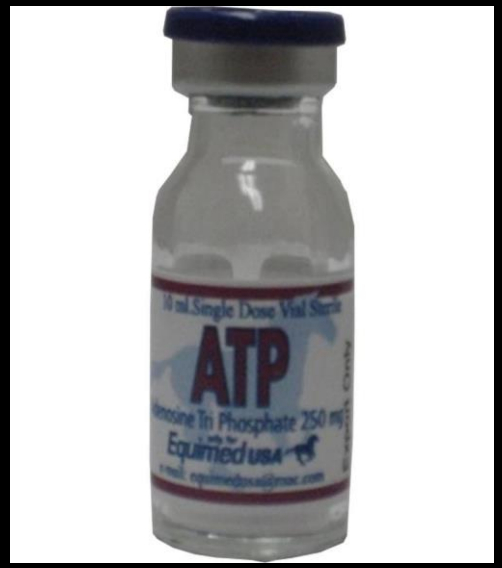
- Adenosine triphosphate (ATP) is considered by biologists to be the energy currency of life, is the molecule associated with **cellular energy in all living cells**.

- Since ATP is present in all living cells, quantifying it enables you to quantify the size of the **total population** rather than only culturable cells.

Energy Carriers

- Adenosine triphosphate, or ATP is a nucleotide that has three phosphate groups.
- Energy is released in a reaction that breaks off the 3rd phosphate group. It's the main energy source for cell processes.





UltraCheck TM 1 Dropper Bottle
1 ng ATP/mL Standard

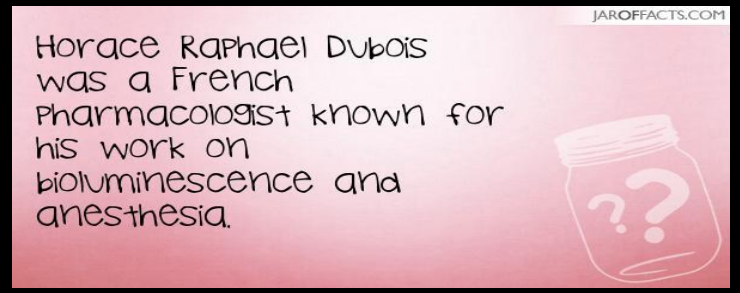
HISTORY OF BIOLUMINESCENCE

- The Greeks and Romans were the first to report the characteristics of luminous organisms. Aristotle (384-322 BC) described 180 marine species and was the first to recognize "cold light." The Greeks also made reference to sea phosphorescence" (about 500 BC)
- In the fifteenth century voyagers commented on the "burning sea" phenomenon. Christopher Columbus (1492) referred to mysterious lights in the water before reaching San Salvador Tropical fireflies in the east were seen by Sir Frances Drake (1540-1596).

- In the sixteenth century references to bioluminescence were found in literature such as Shakespeare (1564 -1616) in Hamlet who talked of the "ineffectual fire" of the glow-worm. English explorers apparently mistook the light from fire beetles for the lights of Spanish campfires and decided to avoid landing on Cuba in 1634, perhaps altering the history of the new world (Jacobs, 1974). The first book devoted to bioluminescence and chemiluminescence was published in 1555 by Conrad Gesner (1555; Carter and Kricka, 1982: Harvey, 1957).

HISTORY OF BIOLUMINESCENCE

- In 1667 Robert Boyle (1667) documented the air requirement for luminescence. Oxygen had not yet been discovered, but now is recognize that this air requirement was, in reality, an oxygen is a requirement of the process.
- This represented a new era in the characterization of bioluminescence rather than just its documentation.
- Early on the nineteenth century Raphael Dubois performed a significant experiment where he extracted the two key components of a bioluminescent reaction and was able to generate light. He coined the terms "luciferin" and the heat labile "luciferase".

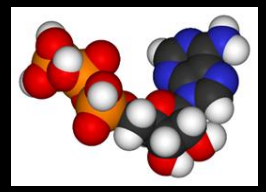


HISTORY OF BIOLUMINESCENCE

- One of the most eminent scientists of the twentieth century was a "Princeton Professor" E.Newton Harvey (1887-1959). He spent much of his life looking for the existence of a luciferin-luciferase system in virtually every luminous organism that he could find (Harvey, 1952). The first luciferin was isolated in 1956 (Green and McElroy, 1956).

HOW DOES IT WORK?

- ATP is recovered from the living cells in a sample through a **chemical extraction**.

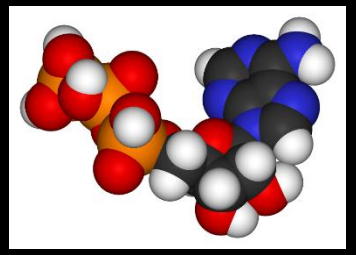


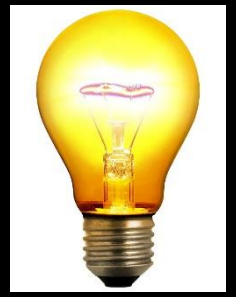
- The **extract** is mixed with a complex reagent containing **Luciferase** to produce **light**.

- The light is measured using a **illuminometer**.

THE REACTION

ATP + Luciferase = Light
Enzyme





- RLU (Relative Light Units) represents an uncalibrated results. Normalizing these results to an ATP standard solution (UltraCheck) corrects for instrument make/model/condition as well as enzyme degradation.
- Converting RLUs into ATP concentrations enable all ATP results to be compared on over time on the same basis.

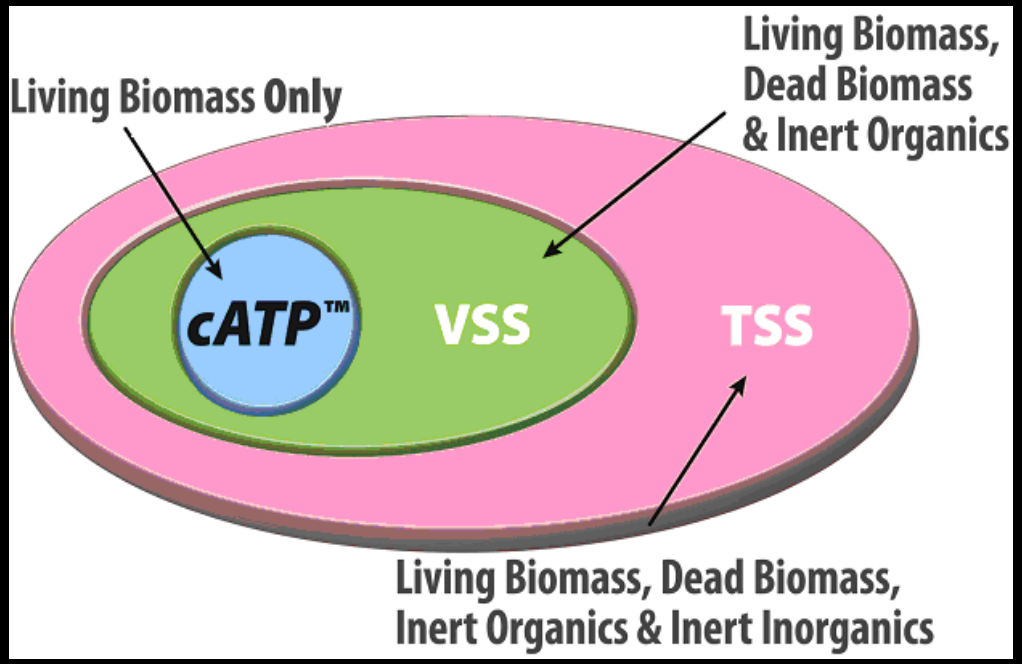
LIMITATIONS

Cannot replace regulatory tests. However, this is not the objective of this tool – it is for operational guidance.

Does not report results in CFU/mL because it does not grow colonies.

Results output in grams of ATP per mL and can be converted to microbial equivalents per mL (this is an estimate of the **number of individual cells per mL**).

WHAT DO ATP TESTS TELL ME?



- **1. Cellular ATP (cATP, ng/mL):** Direct indication of **living biomass population**
- **2. Biomass Stress Index (BSI, %):** the **stress level** of the microorganisms, toxicity (Dissolved ATP/ Total ATP)
- **3. Active Biomass Ratio (ABR, %):** Percentage of **solids inventory that is alive** (with TSS data)

FORMS OF ATP

- The QG21W protocol involves two tests per sample.
- Total ATP (tATP) – Measures ATP extracted from live cells as well as that which was already present from cells that recently died or are stressed.
- Dissolved ATP (dATP) – ATP from dead and stressed cells.

- Cellular ATP (cATP): present in living, active cells
- Dissolved ATP (dATP): extracellular, released from dead and dying/stressed microorganisms

- **Total ATP = cATP + Complexed ATP + Dead/Dying biomass + free ATP (soluble)**

- Measured by dATP using recovery reagent LumiSolve

ATP TARGETS (QGA)

* ATP analyses isolate and quantify the **live microbiological content** in water.

* This data can be compared to recommended targets for finished water to assess the effectiveness of microbiological control programs and manage risk:

Good Control: ATP < 0.5pg/mL

Moderate Risk: 0.5 < ATP < 10pg/mL

High Risk: ATP > 10pg/mL

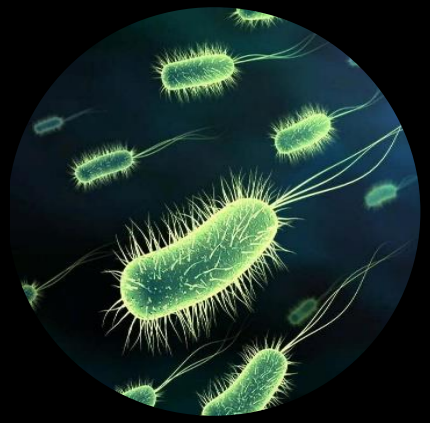
STORAGE TANKS

Storage tanks is often the first point at which regrowth becomes a problem.

Whether it be due to long water age, stagnation, or infiltration, stored water represents a threat to downstream water quality.

$[ATP]_{outlet} > [ATP]_{inlet}$? Growth is occurring in the tank.

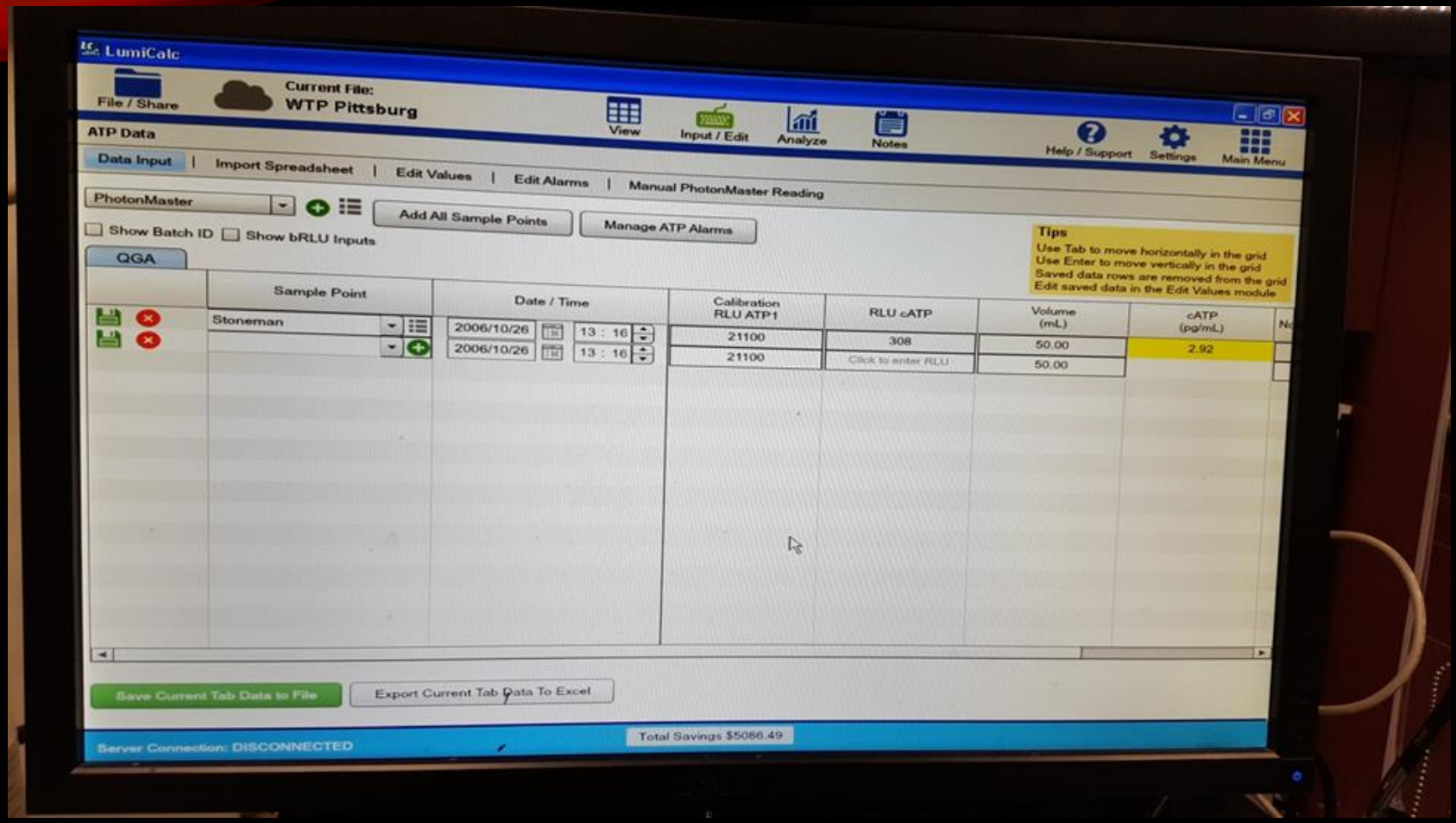
TOTAL COLIFORM RULE

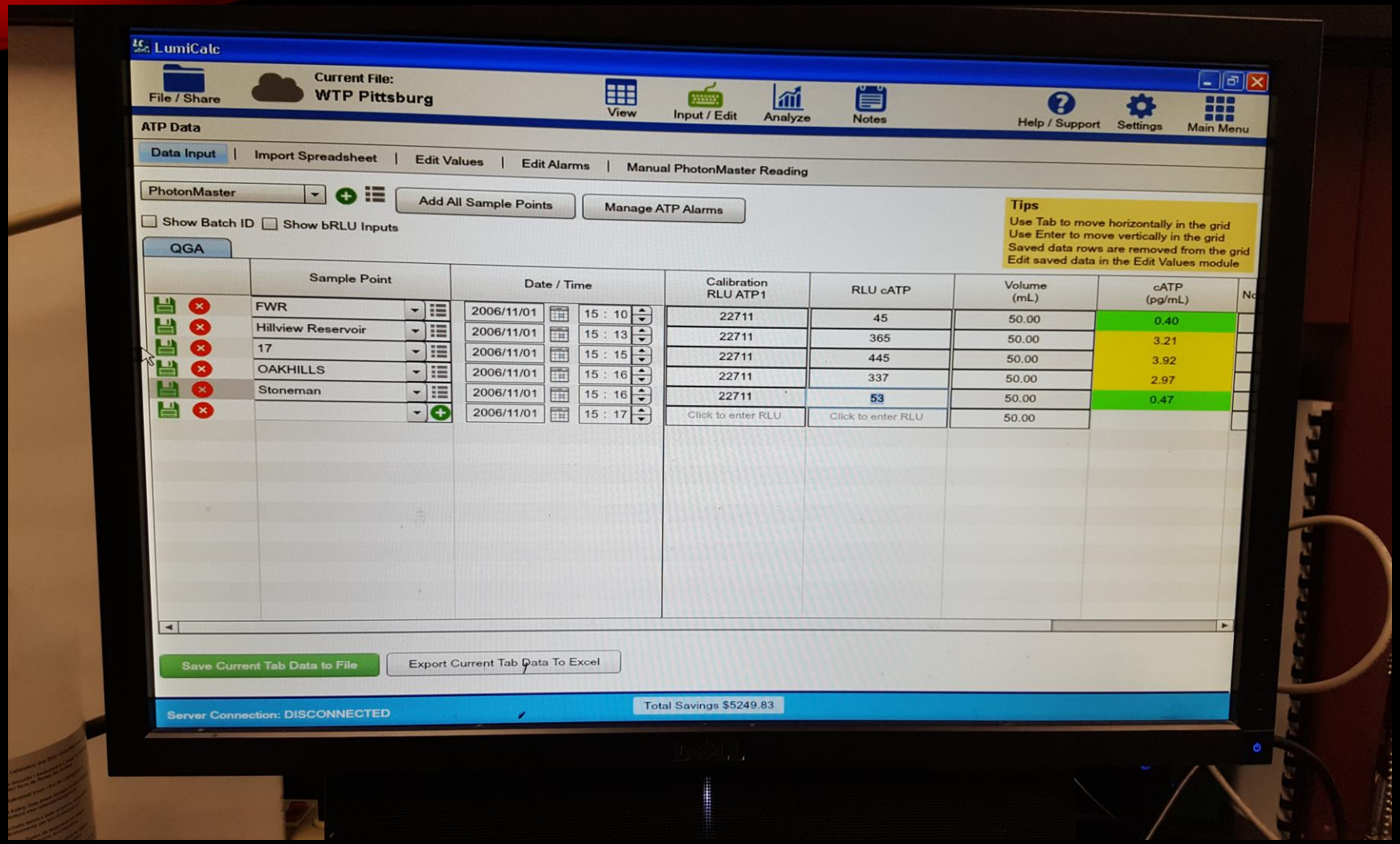


- In many cases, microbiological tests done by water utilities are limited to compliance tests (e.g. Total Coliform).

Non-Pathogenic microbes can cause several issues within a system:

- They will **consume disinfectant** residual (which influences TCR since residual must be maintained)


LumiCalc
File / Share
Current File:
WTP Pittsburg
View
Input / Edit
Analyze
Notes
Help / Support
Settings
Main Menu
ATP Data
Data Input
Import Spreadsheet
Edit Values
Edit Alarms
Manual PhotonMaster Reading
PhotonMaster
Add All Sample Points
Manage ATP Alarms
Show Batch ID
Show bRLU Inputs
QGA
Tips
Use Tab to move horizontally in the grid
Use Enter to move vertically in the grid
Saved data rows are removed from the grid
Edit saved data in the Edit Values module
Sample Point
Date / Time
Calibration RLU ATP1
RLU cATP
Volume (mL)
cATP (pg/mL)
Stoneman
2006/10/26
13 : 16
21100
308
50.00
2.92
2006/10/26
13 : 16
21100
Click to enter RLU
50.00
Save Current Tab Data to File
Export Current Tab Data To Excel
Server Connection: DISCONNECTED
Total Savings $5086.49


LumiCalc
File / Share
Current File:
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View
Input / Edit
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Main Menu
ATP Data
Data Input | Import Spreadsheet | Edit Values | Edit Alarms | Manual PhotonMaster Reading
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Show Batch ID
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Tips
Use Tab to move horizontally in the grid
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QGA
Sample Point
Date / Time
Calibration RLU ATP1
RLU cATP
Volume (mL)
cATP (pg/mL)
FWR 2006/11/01 15 : 10 22711 45 50.00 0.40
Hillview Reservoir 2006/11/01 15 : 13 22711 365 50.00 3.21
17 2006/11/01 15 : 15 22711 445 50.00 3.92
OAKHILLS 2006/11/01 15 : 16 22711 337 50.00 2.97
Stoneman 2006/11/01 15 : 16 22711 53 50.00 0.47
2006/11/01 15 : 17 Click to enter RLU Click to enter RLU 50.00
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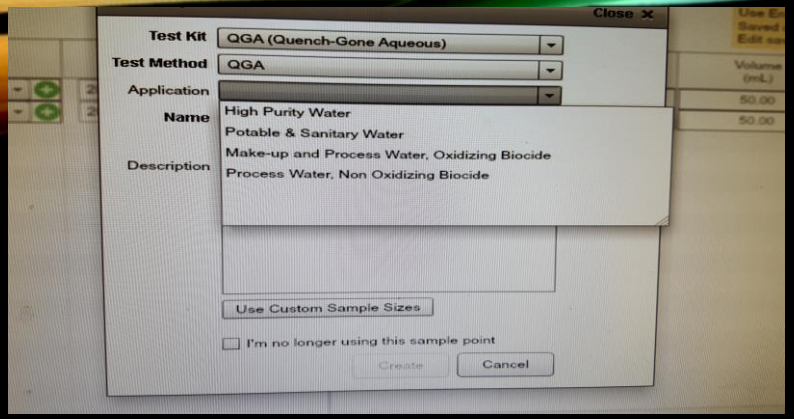
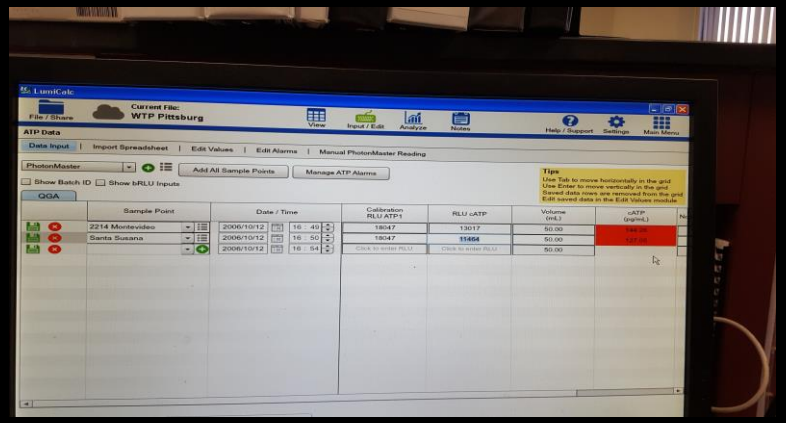
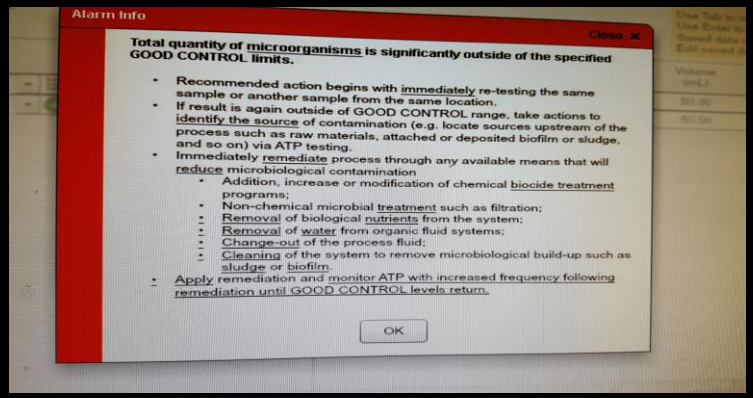
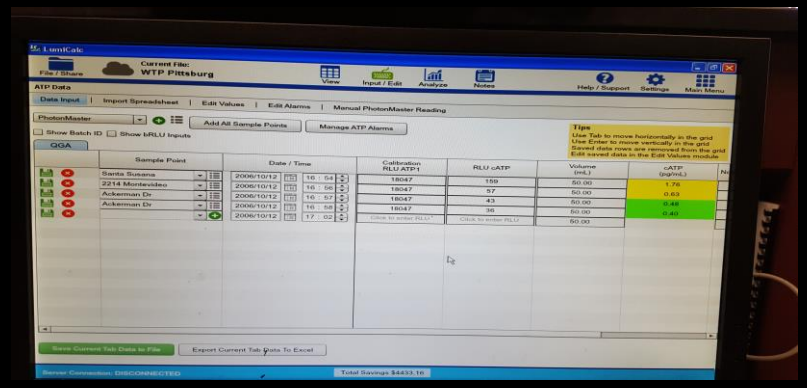
Total Savings $5249.83


Close
Test Kit
QGA (Quench-Gone Aqueous)
Test Method
QGA
Application
Name
Description
High Purity Water
Potable & Sanitary Water
Make-up and Process Water, Oxidizing Biocide
Process Water, Non Oxidizing Biocide
Use Custom Sample Sizes
I'm no longer using this sample point
Create
Cancel


LumiCalc
Current File:
WTP Pittsburg
ATP Data
Data Input
Import Spreadsheet
Edit Values
Edit Alarms
Manual PhotonMaster Reading
Add All Sample Points
Manage ATP Alarms
Tips
QGA
Sample Point
Date / Time
Calibration RLU ATP1
RLU cATP
Volume (mL)
cATP (ng/mL)
2214 Montevideo
Santa Susana


Alarm Info
Close
Total quantity of microorganisms is significantly outside of the specified GOOD CONTROL limits.
Recommended action begins with immediately re-testing the same sample or another sample from the same location.
If result is again outside of GOOD CONTROL range, take actions to identify the source of contamination (e.g. locate sources upstream of the process such as raw materials, attached or deposited biofilm or sludge, and so on) via ATP testing.
Immediately remediate process through any available means that will reduce microbiological contamination
Addition, increase or modification of chemical biocide treatment programs;
Non-chemical microbial treatment such as filtration;
Removal of biological nutrients from the system;
Removal of water from organic fluid systems;
Change-out of the process fluid;
Cleaning of the system to remove microbiological build-up such as sludge or biofilm.
Apply remediation and monitor ATP with increased frequency following remediation until GOOD CONTROL levels return.
OK


LumiCalc
Current File:
WTP Pittsburg
ATP Data
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Import Spreadsheet
Edit Values
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Add All Sample Points
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Tips
QGA
Sample Point
Date / Time
Calibration RLU ATP1
RLU cATP
Volume (mL)
cATP (ng/mL)
Santa Susana
2214 Montevideo
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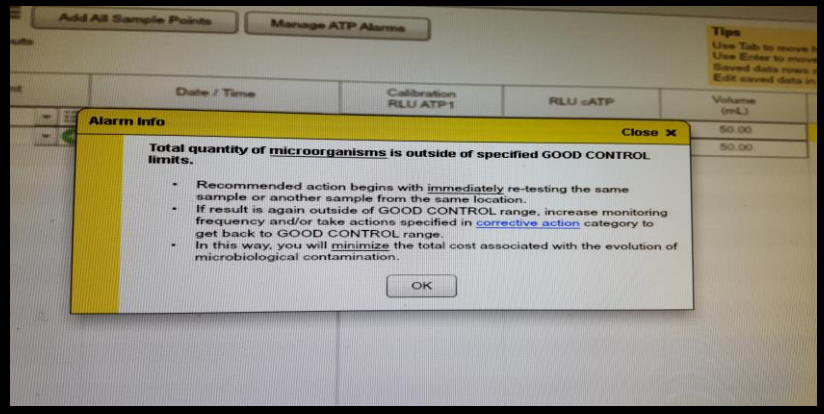
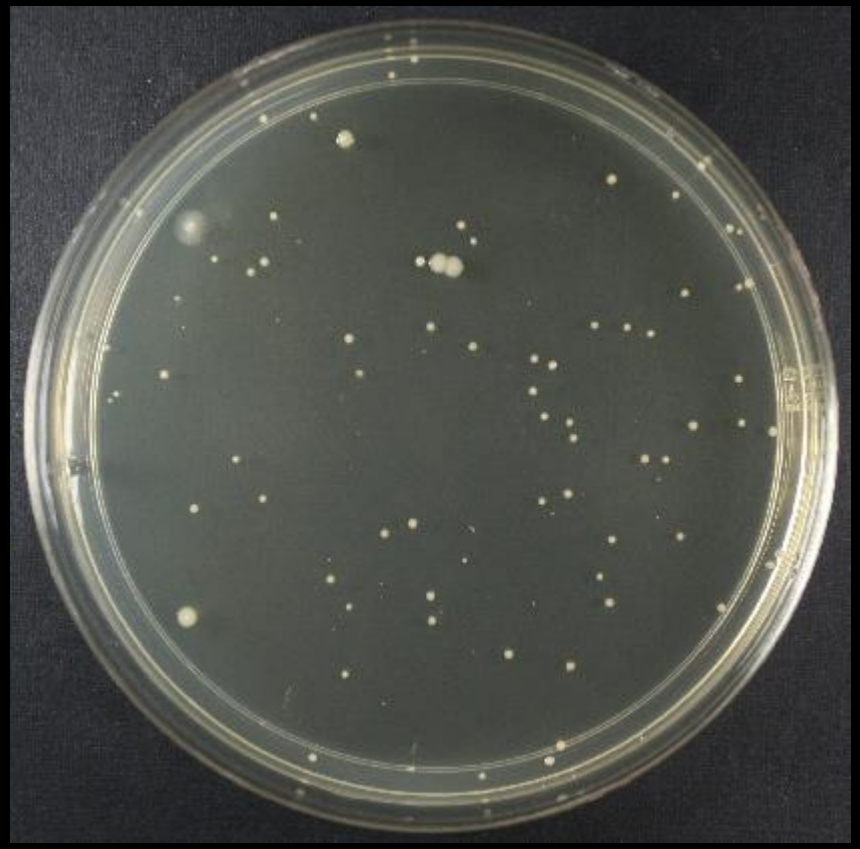
Ackerman Dr
Save Current Tab Data to File
Export Current Tab Data To Excel


Add All Sample Points
Manage ATP Alarms
Tips
Date / Time
Calibration RLU ATP1
RLU cATP
Volume (mL)
Alarm Info
Close
Total quantity of microorganisms is outside of specified GOOD CONTROL limits.
Recommended action begins with immediately re-testing the same sample or another sample from the same location.
If result is again outside of GOOD CONTROL range, increase monitoring frequency and/or take actions specified in corrective action category to get back to GOOD CONTROL range.
In this way, you will minimize the total cost associated with the evolution of microbiological contamination.
OK

HETEROTROPHIC PLATE COUNT (HPC)



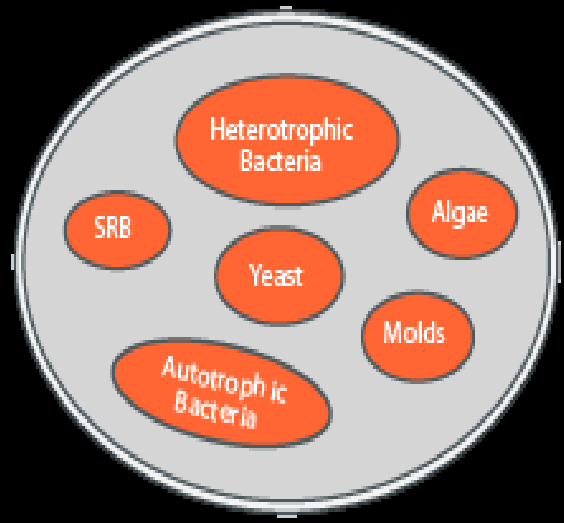
- HPC is an attempt at a total microbial count (CFU/mL).
- Requires at least 48 hours.
- Luminultra require only 5 min

Correlation

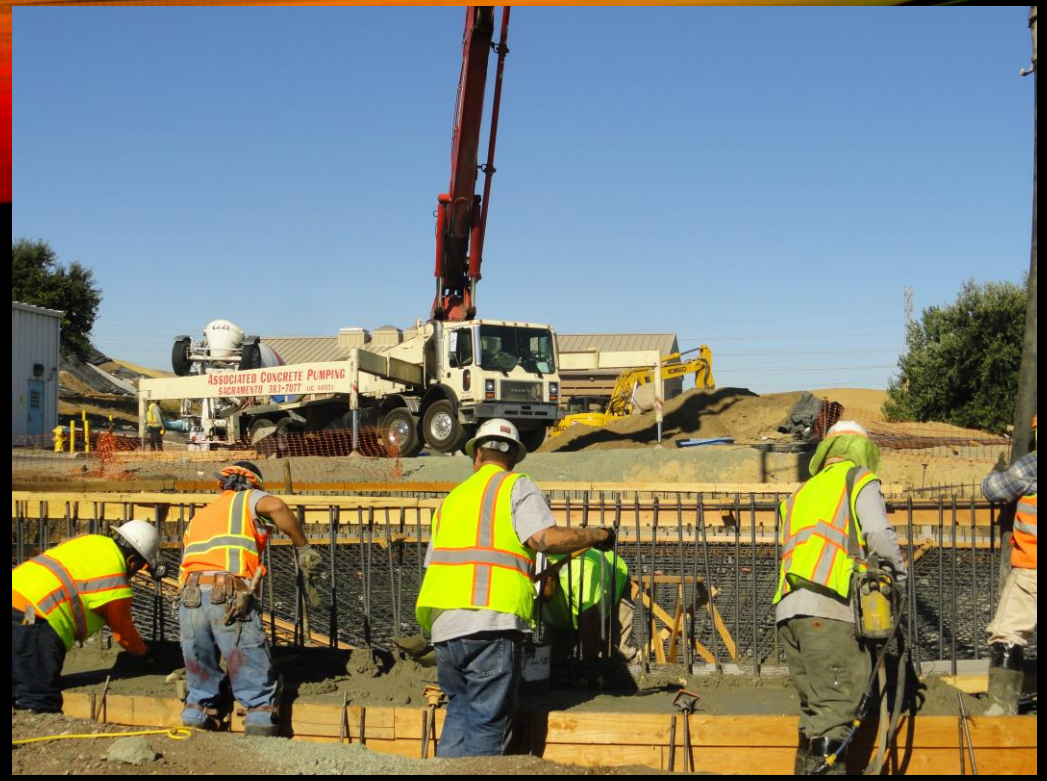
- 1.20 c ATP pg/mL
- Coliform = negative
- HPC = 19 CFU
- Turbidity 0.105
- Chlorine Residual = 0.1 mg/L

READINGS ON DISTRIBUTION

13.18 cATP pg/mL
Coliform = positive
HPC = 25 CFU
Turbidity 0.141
Chlorine Residual = 0.2 mg/L
After flushing 0.81 cATP pg/mL

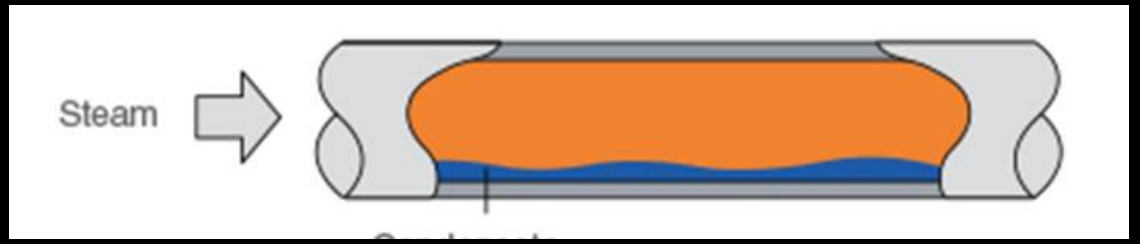


44.42 cATP pg/mL
Coliform = negative
HPC = 28 CFU
Turbidity 0.130
Chlorine Residual = 0.2 mg/L
After flushing 1.3 cATP pg/mL

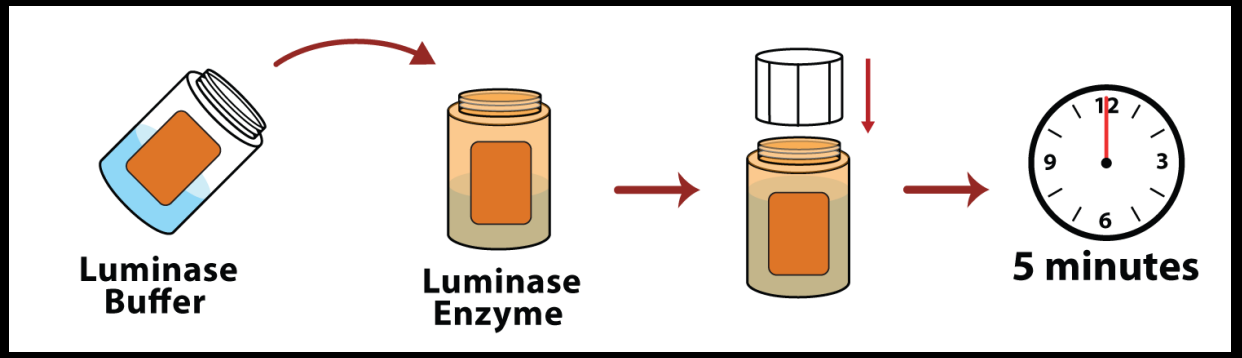

ASSOCIATED CONCRETE PUMPING
SACRAMENTO 383-7077


1
2
3

ACCUMULATION IN WATER MAINS

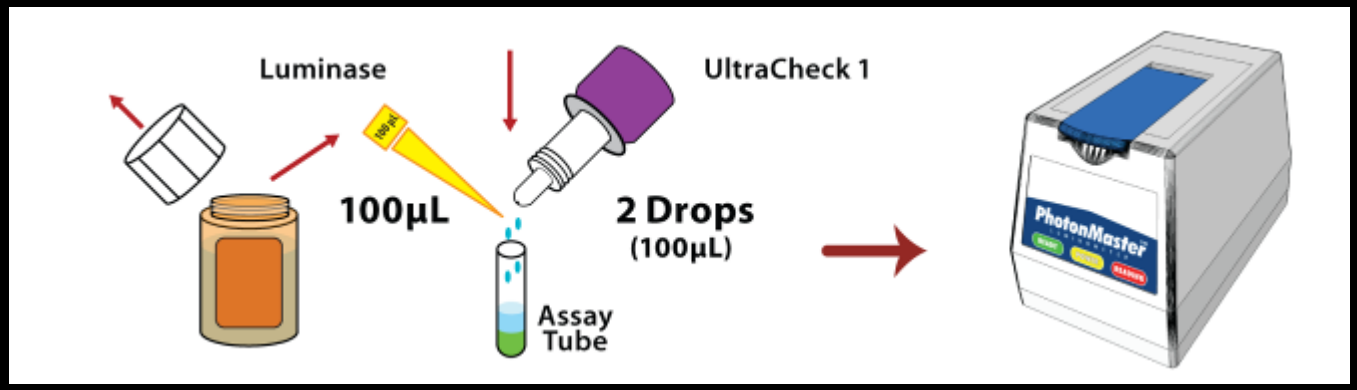


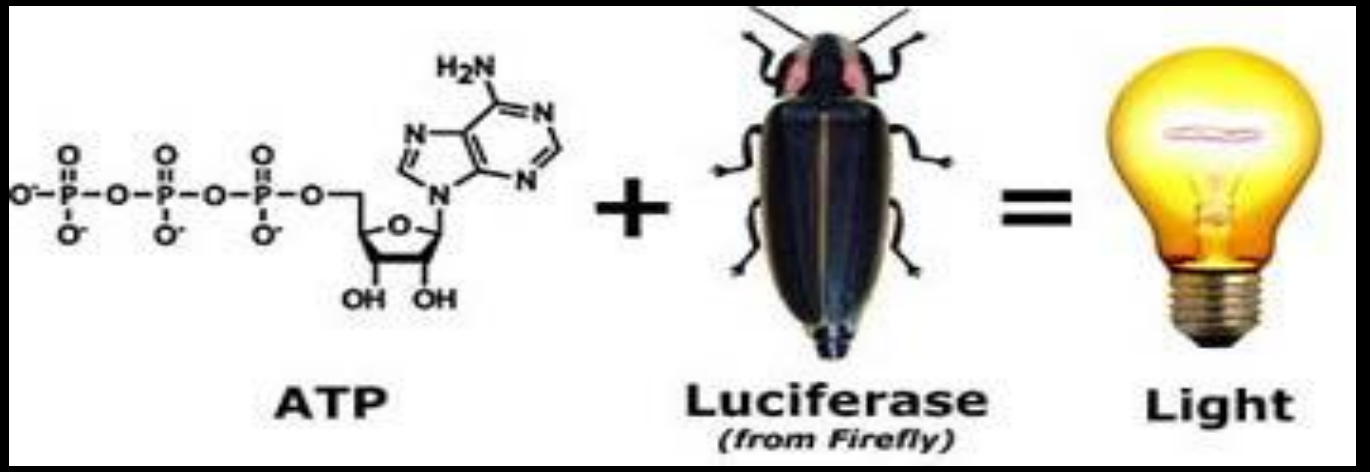
WORKING WITH LUMINULTRA



ATP STANDARD CALIBRATION

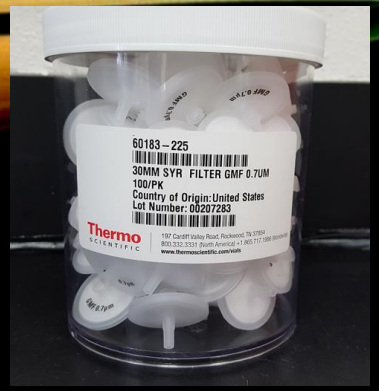
ULTRACHECK 1 DROPPER BOTTLE (1 NG ATP/ML STANDARD)





A newly rehydrated vial of Luminase should result in a calibration RLU value of approximately **15,000 – 25,000 RLU**.

If the reading is less than **5,000 RLU**, Luminase activity is too low and it is best to rehydrate a new vial for optimum sensitivity.



REAGENTS

(TEST KIT QGA)

LUCIFERASE

LuminaseT Enzyme & Buffer Vials

LUCIFERASE

SURFACTANT

UltraLyse TM 7 Bottle

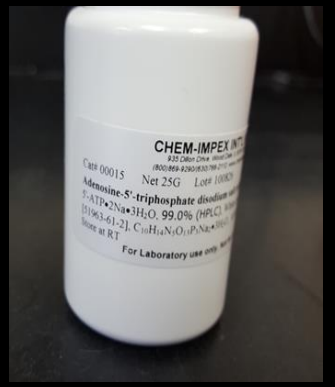
ATP Extraction Reagent, 125mL

(Surfactant)

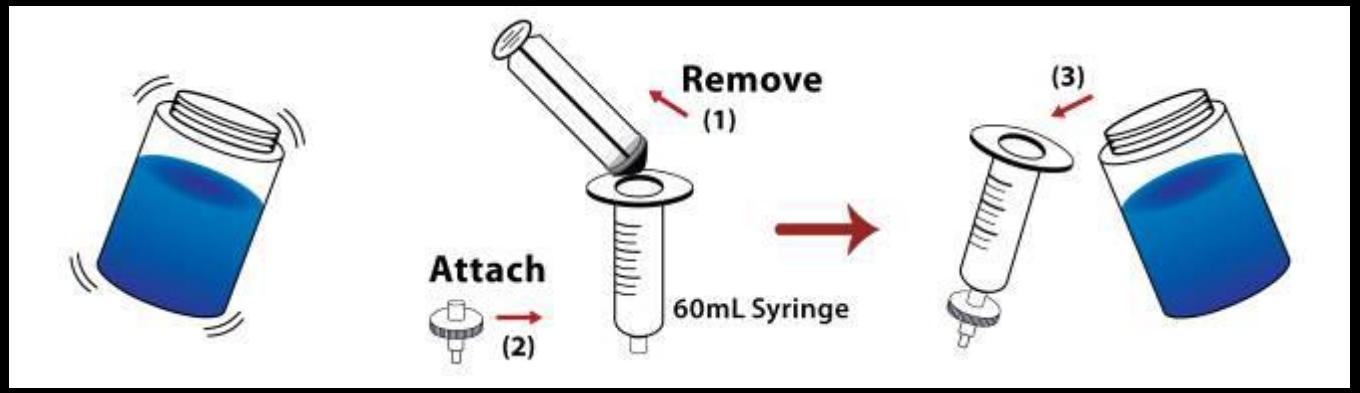
ATP

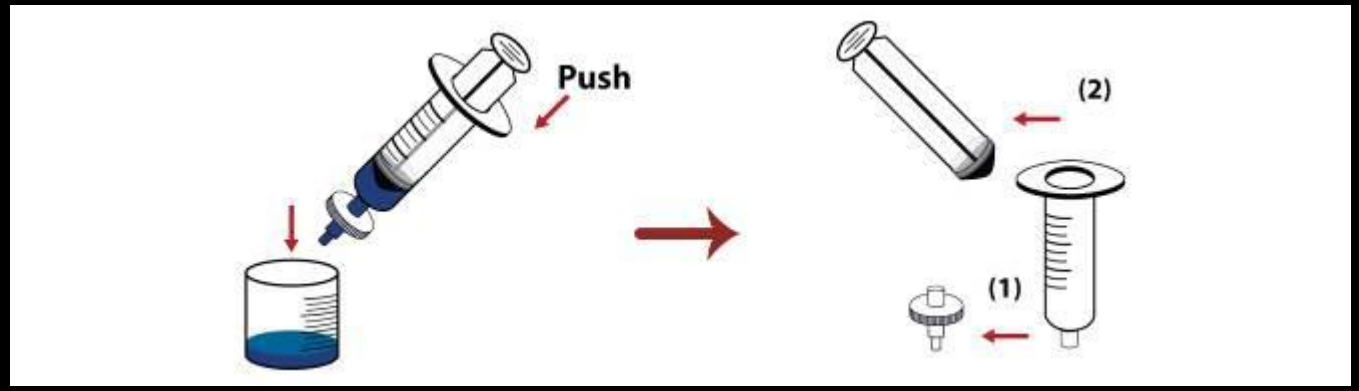
UltraCheck TM 1 Dropper Bottle

1 ng ATP/mL Standard

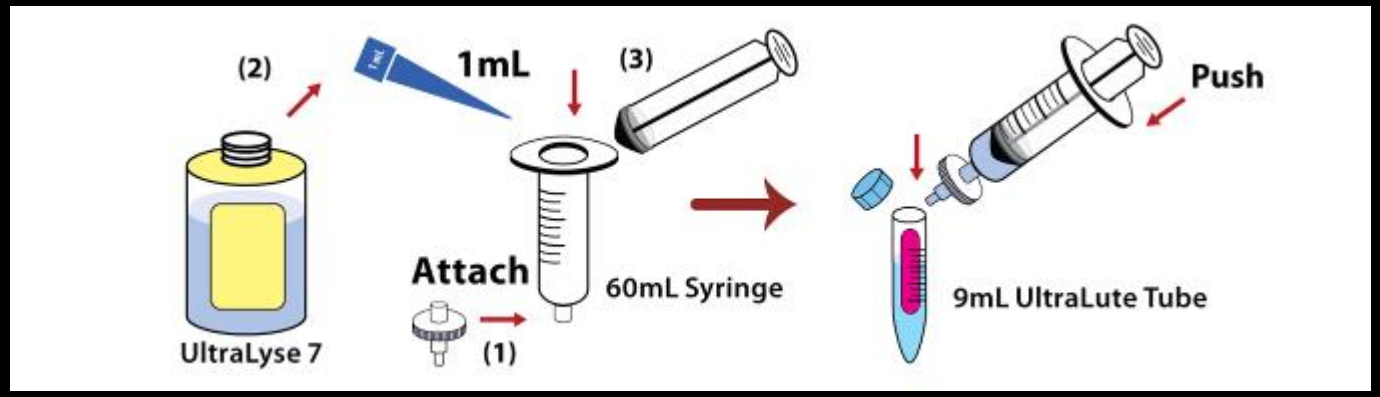


FILTRATION



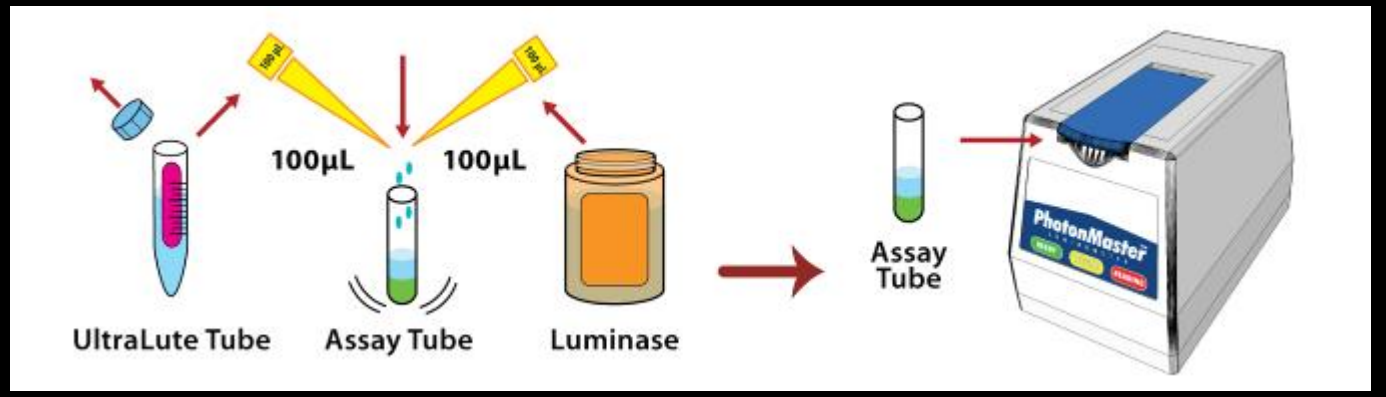


EXTRACTION


(2)
1mL
(3)
Push
Attach
60mL Syringe
9mL UltraLute Tube
UltraLyse 7
(1)

READING


ATP
Luciferase
(from Firefly)
Light


100µL
100µL
UltraLute Tube
Assay Tube
Luminase
Assay
Tube
PhotonMaster

$$cATP\left(pg\ ATP/mL\right) = \frac{RLU_{cATP}}{RLU_{UC1}} \times \frac{10{,}000}{V_{Sample}\ (mL)}$$

QGA CALCULATIONS

Cellular ATP (cATP) is the ATP found in living cells and represents the amount of live microbial contamination in the sample.

$$cATP\left(\frac{pgATP}{ml}\right) = \frac{RLU_{cATP}}{RLU_{UC1}} \times \frac{10{,}000}{V_{Sample(mL)}}$$

QGA tests utilize **filtration** to concentrate microbes and indicate **total cellular ATP levels.**

QGA CALCULATIONS

ATP results can be converted to **microbial equivalents** using an approximate estimation of the amount of ATP per average-sized cell.

$$Microbial\ Equivalents\left(\frac{\#}{ml}\right) \quad = \quad cATP\left(\frac{pg}{ml}\right) \ x \ \ 1{,}000$$

Conversion is performed assuming the average cell size is roughly equivalent to a typical E.Coli (1fg or 10^{-3}pg ATP). 1fg = 10^{-15}g; 1pg = 10^{-12}g

DATA INTERPRETATION

Application	Good Control (cATP, pg/mL)	Moderate Risk (cATP, pg/mL)	High Risk (cATP, pg/mL)
High-Purity	< 0.1	0.1 – 1.0	> 1.0
Potable Water	< 0.5	0.5 – 10	> 10
Cooling Water	< 10	10 – 100	> 100
Sessile Build-up*	< 10x	10x – 100x	> 100x

Information is power!
More knowledge you have about the total microbial threat.
More proactive your solutions can be.


THANKS