Evaluation of the ATP Test for Nitrification Monitoring at the Portland Water Bureau

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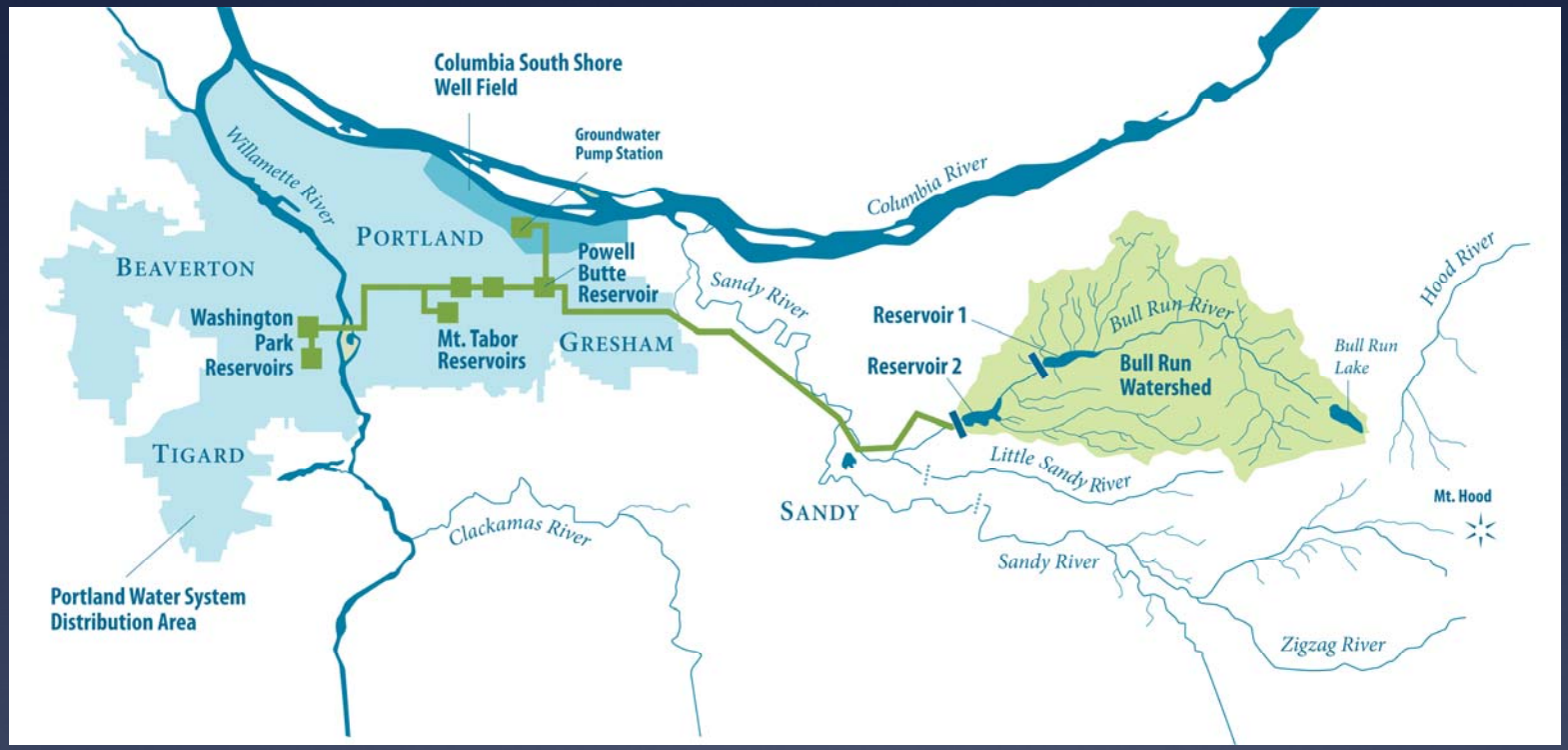
PORTLAND WATER BUREAU

FROM FOREST TO FAUCET

Presentation Outline

- Background
  - PWB System
  - Nitrification Monitoring at the PWB
- ATP Evaluation
- Case Study - Bertha
- Next Steps

Portland System - Overview



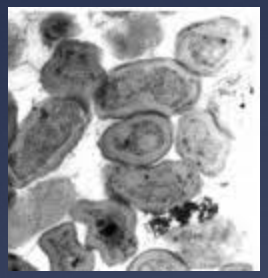
- Serves approximately 938,000 people
- Retail and wholesale service area is approximately 225 square miles
- 20 wholesalers which comprise approximately 42% of our demand

Portland's Distribution System

- ~ 180 pressure zones
- ~70 storage tanks
- 39 pump stations
- > 2200 miles of distribution pipeline
  - More than 200 miles of which are 16" in diameter or larger
- 3 large uncovered finished water reservoirs that are routinely in service
  - Res 1 = 12 MG
  - Res 3 = 16 MG
  - Res 5 = 49 MG
- Over 220 water quality sample stations

Nitrification Review

- Nitrification is the bacteriological oxidation of ammonia to nitrate via nitrite
  - 1st Step: ammonia to nitrite



$+ NH_3 + O_2 \rightarrow NO_2^- + 3H^+ + 2e^-$

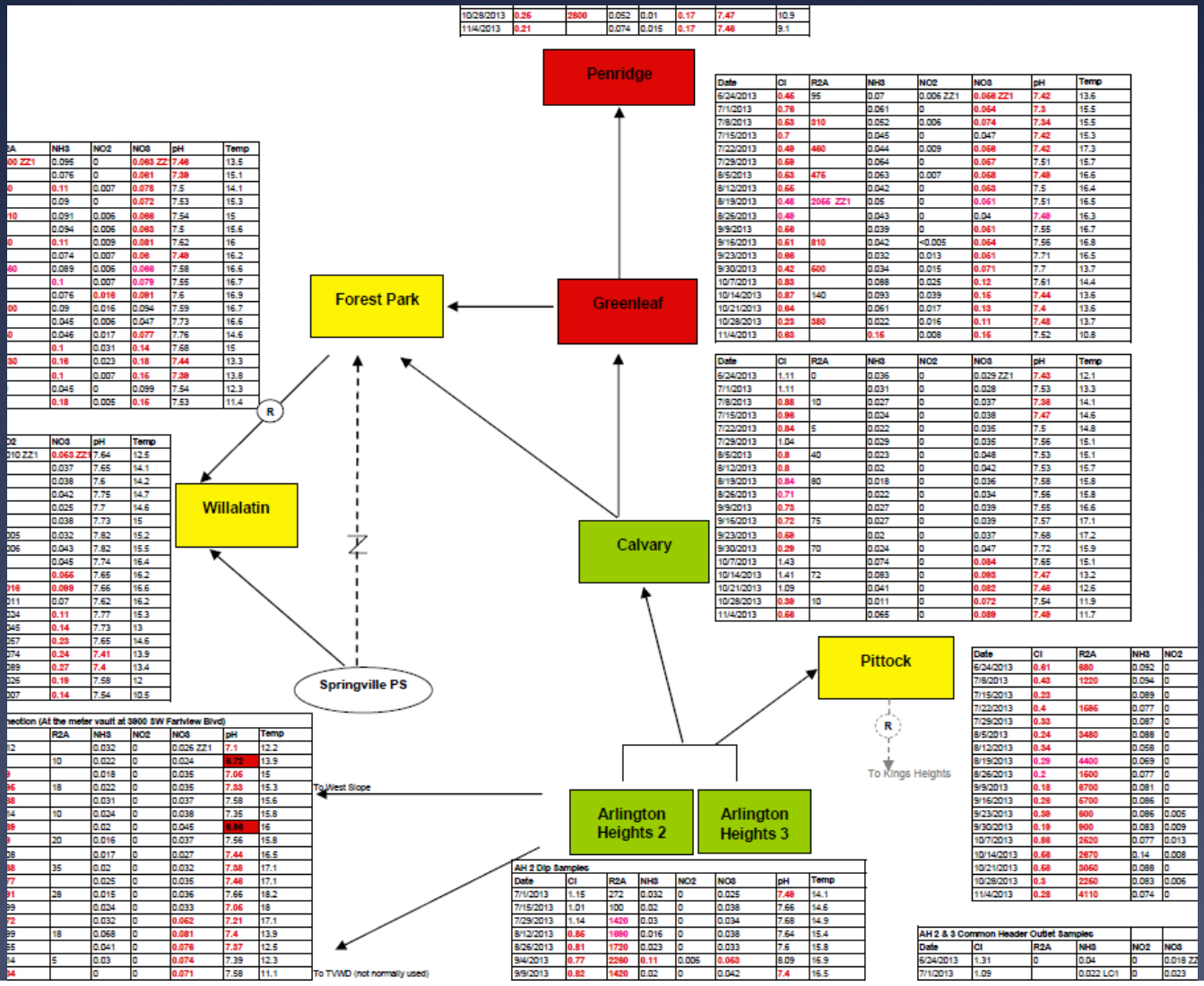
  - 2nd Step: nitrite to nitrate (complete nitrification)

$+ NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$

Historic Nitrification Monitoring at PWB

- 1999-2000 Study
  - Determined that we see nitrification and the season can extend into the late fall
- 2011 Tank Monitoring Study
  - Monitored chlorine, free ammonia, temperature, nitrite and nitrate at a subset of tanks
- 2012 Study
  - TCR sites
  - If pH or chlorine residual fell below chronic limits in two consecutive samples or acute limits in one sample, the site qualified for nitrification monitoring

2013 Nitrification Program Goal – More Holistic Approach



2013 Study - Sampling locations

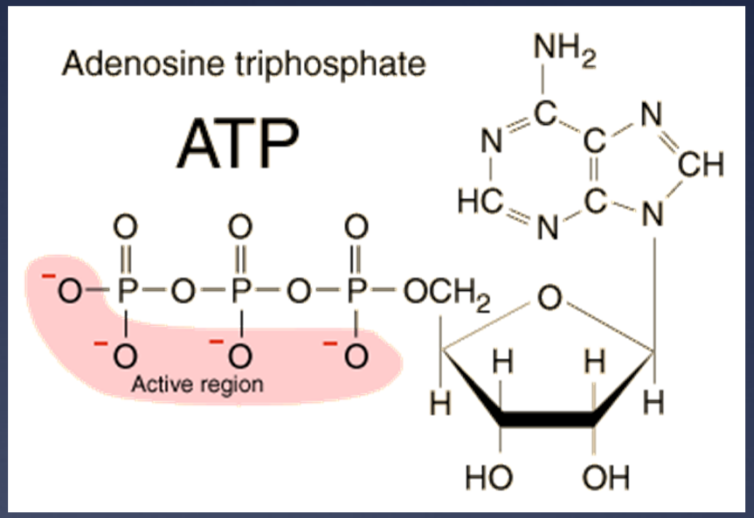
- Powell Butte = hub of the system
- Select TCR sites (23)
- Storage tanks (20)
- Open reservoirs
- Dead-ends/problematic areas in the system (2)
- Other areas not represented by other sampling events (4)
- Wholesaler connections (4)

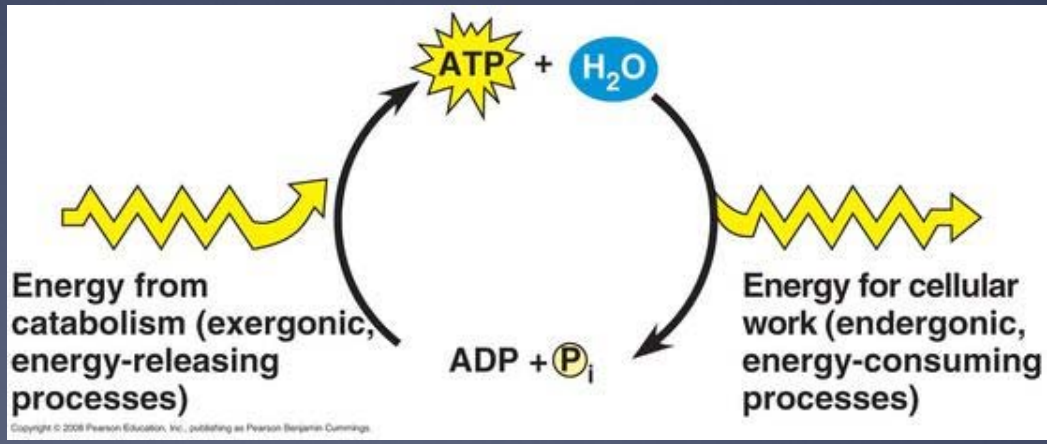
Parameters Monitored

- Lab (results available 24 hours – 7 days)
  - Nitrite
  - Nitrate
  - Free ammonia
  - R2A-HPC
- Field (results available same day)
  - Chlorine residual
  - pH
  - Temp
  - ATP

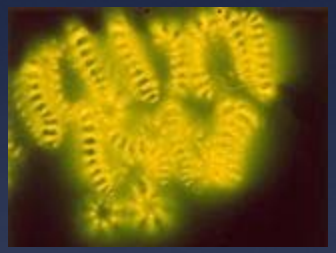
ATP Basics

- ATP = Adenosine Triphosphate
  - Discovered in 1929
  - Universal energy carrier in all living organisms
  - It is present in every cell; energy from the breakdown of ATP drives many important reactions in the cell.
  - Believed that there is a good correlation between cellular ATP and the number of viable bacteria present

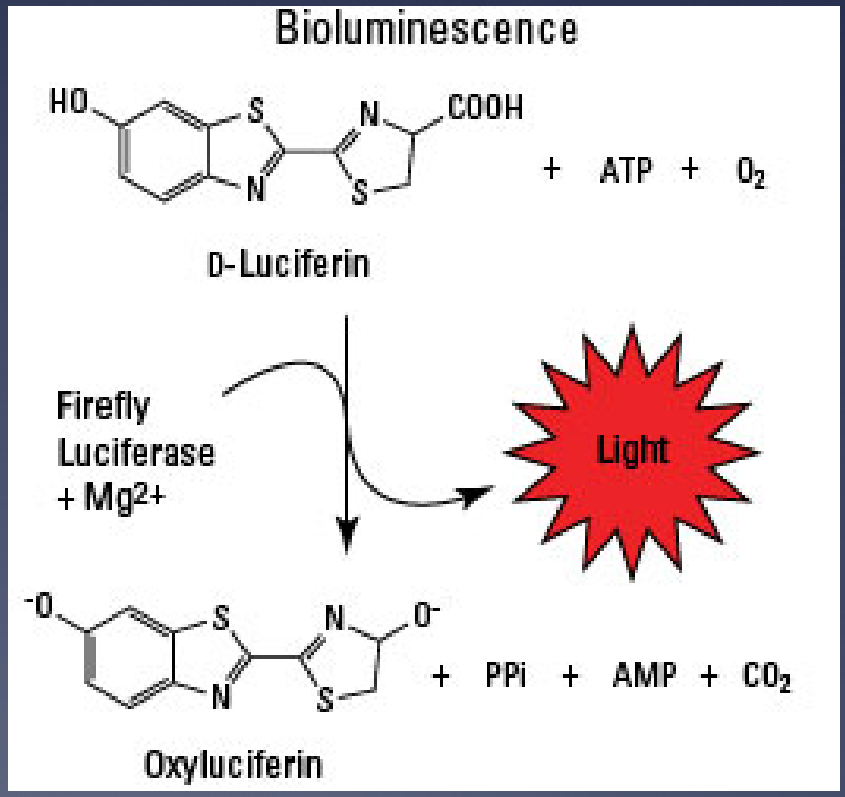




ATP Analysis



- ATP analysis is not new
  - Has been used for decades in other fields such as medical research, food hygiene, wastewater etc.
- Had not been used as widely in drinking water because it was a difficult test and we had little information regarding the average ATP concentrations in natural bacteria
- ATP analysis works by measuring bioluminescence
  - Bioluminescence is light produced within a living organism; often enzyme catalyzed
- ATP + luciferase = light



Why Evaluate ATP?

- HPC-R2A is a heterotrophic plate count test that uses R2A agar instead of standard agar
  - This nutrient poor media allows nitrifiers to preferentially grow
  - It is considered a good early warning indicator of nitrification in drinking water
  - Time-consuming test that does not provide results for seven days
- ATP can provide results the same day
- Objective of this study was to compare HPC-R2A results with those from an ATP field kit
  - Basically, is ATP a good early warning indicator for nitrification?

Test Kits Evaluated

- 2 methods were compared:
  - 3M: Clean Trace Water Test
  - Luminultra: Quench-Gone Aqueous (QGA) Test

Reagents and Equipment

- Luminultra
  - Reagents
    - ATP source (water sample)
    - ATP standard (Ultracheck)
    - Lysing agent (UltraLyse)
    - Dilution reagent (UltraLute)
    - Luciferase enzyme (Luminase)
  - Equipment
    - Sterile bottle for the sample
    - Syringe and filter
    - Test tubes
    - Pipettes (1mL and 100 uL) and pipette tips
    - Luminometer
- 3M
  - Reagents
    - ATP source (water sample)
    - Swabs contain all reagents
      - Total ATP swab
      - Free ATP swab
  - Equipment
    - Sterile bottle for the sample
    - Luminometer

3M Test Methodology

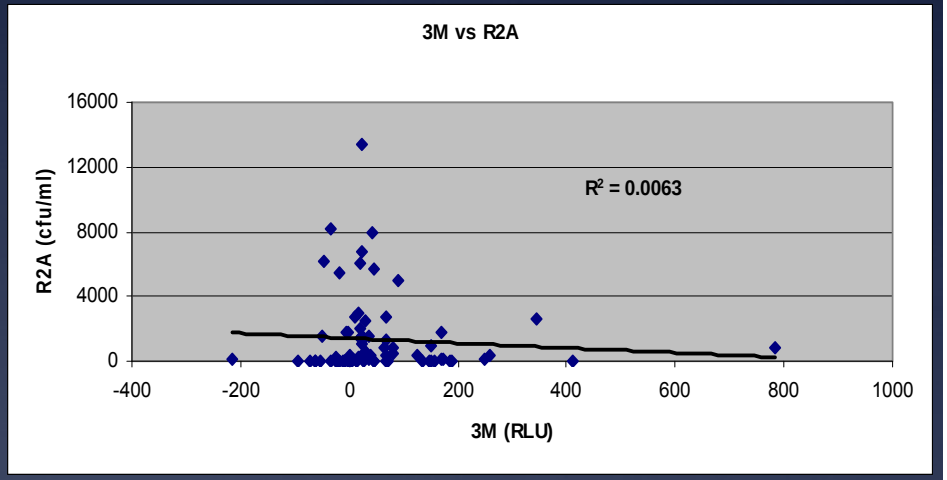
- The 3M test measures the live microbial load in a sample
- Free ATP is measured in the sample with a free ATP swab
- Total ATP is measured in the sample using a total ATP swab
- Total – Free = Live Microbial Load (in RLUs)

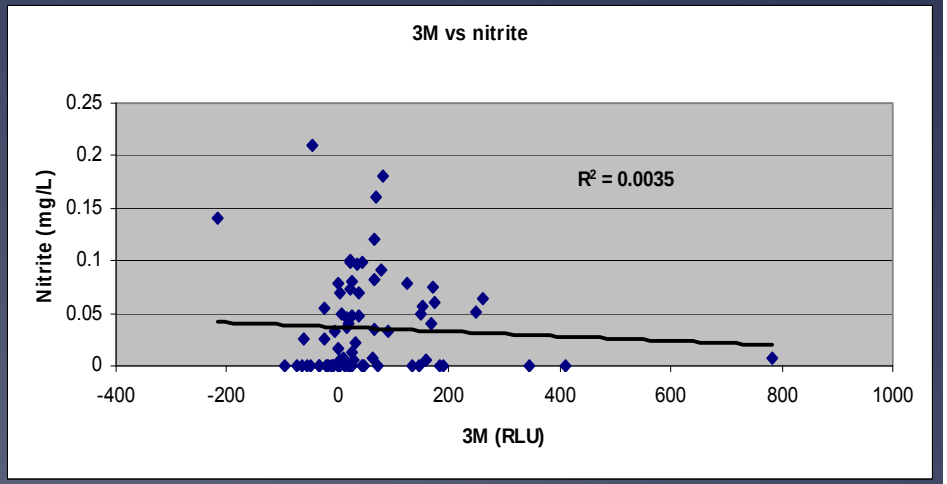
3M Procedure

- Step 1: Test free ATP
  - Immerse the swab into the sample up to the top of the sampling rings– DO NOT swirl
  - Tap the handle of the sample stick to dislodge any bubbles
  - Remove any excess drops that may have formed on the bottom of the swab
  - Immediately insert the swab back into the pen and push the plunger completely down to insert the swab into the reagent
  - Shake vigorously from side to side
  - Insert into luminometer
- Step 2: Test total ATP
  - Repeat above steps

3M Results

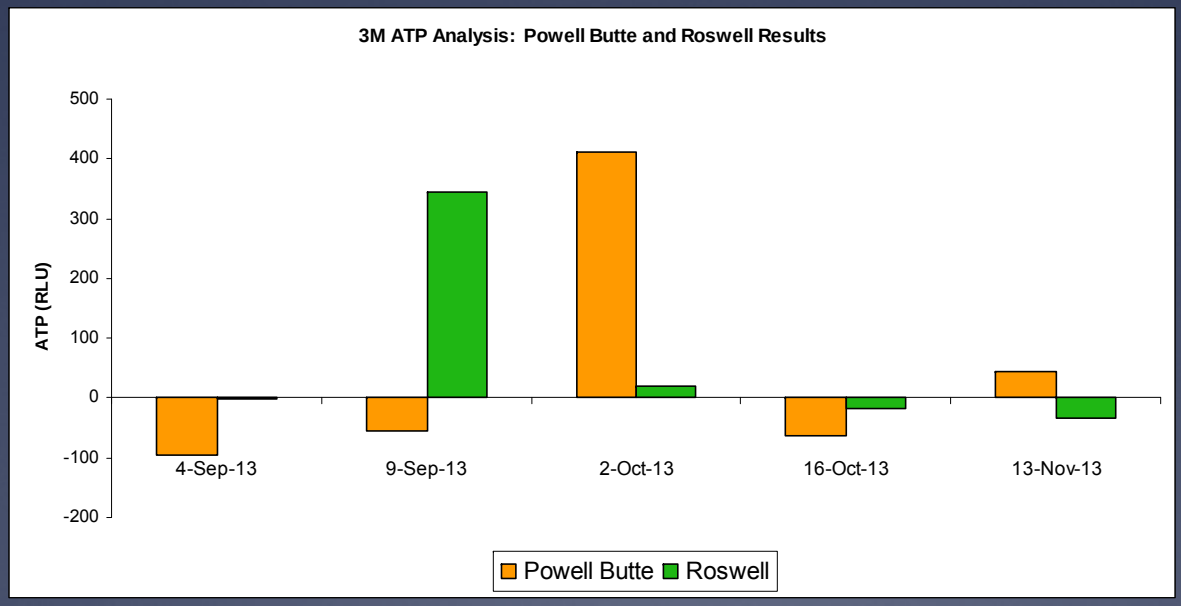
- Did not relate to nitrification parameters in our water
- Compared ATP results to R2A, nitrite, nitrate, chlorine
  - R^2 values ranged from 0.0006 - 0.0063
  - No relationship between these parameters





3M Results Continued

- Did not correlate to overall water quality
  - Powell Butte (best water)
  - Roswell (worst water)
- The test did not discern between the two sites
- Wide range of values observed in Powell Butte despite the fact that WQ is relatively constant
- Some results were negative
- Unfortunate because of ease of test and responsiveness of vendor



Luminultra Quench Gone Aqueous Test Methodology

* The QGA test measures ATP from living cells only
* The raw results from the analysis are in units of Relative Light Units (RLUs), which are then converted to cellular ATP (cATP) according to the following equation:

$$cATP\,(pg\,ATP/mL) = \frac{RLU_{cATP}}{RLU_{ATP1}} \times \frac{10{,}000\,(pg\,ATP)}{V_{Sample}\,(mL)}$$

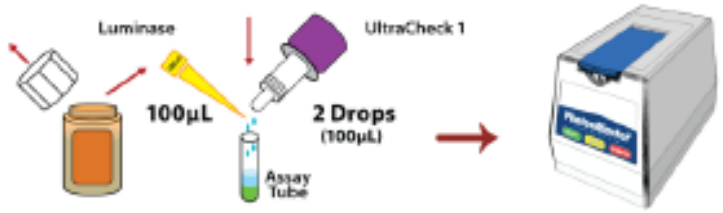
* cATP represents ATP from living microorganisms and is a direct indication of total living biomass quantity.

Luminultra QGA Test Procedure

- Step 1: Standard calibration – perform one calibration per day
  - Add 2 drops of standard to 100 uL luminase and read in luminometer

Step 1 - UltraCheck™ 1 Calibration

Perform one UltraCheck 1 calibration per day or per each set of samples analyzed.

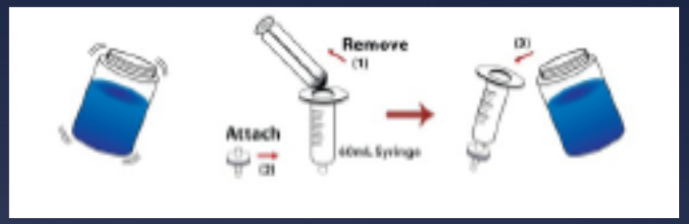


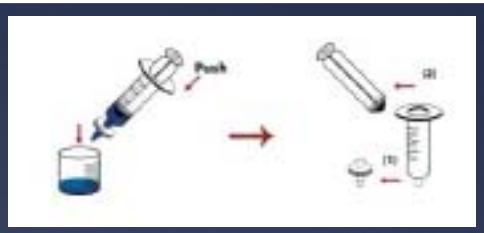
NOTE: If RLU_{ATP1} ≤ 5,000 using a PhotonMaster or Lumitester C-110, rehydrate a new bottle of Luminase for maximum sensitivity.

*Adapted from Luminultra http://www.luminultra.com/files/QGA_Quick_Reference_Guide_EN.pdf

Luminultra QGA Test Procedure

- Step 2: Sample analysis
  - Filter sample – use a syringe with a 0.7 um filter (sample size of 100 mL is best for drinking water)
  - Run 1 mL of lysing solution through the filter to extract ATP
  - Collect this filtrate in a dilution tube
    - This is stable at room temp for 4 hours
  - Pipette 100 uL of dilution tube solution into a test tube
  - To this, add 100 uL of the enzyme, luminase
  - Swirl five times and read in a luminometer





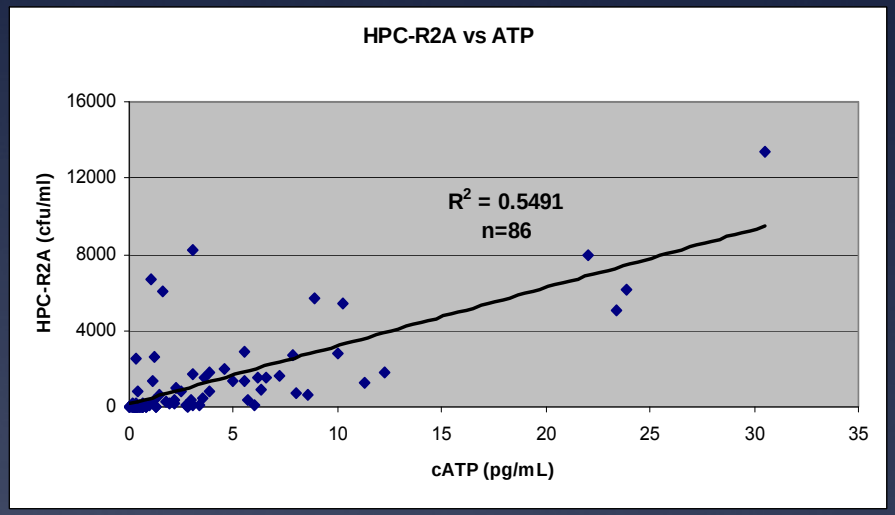
*Adapted from Luminultra
http://www.luminultra.com/files/QGA_Quick_Reference_Guide_EN.pdf

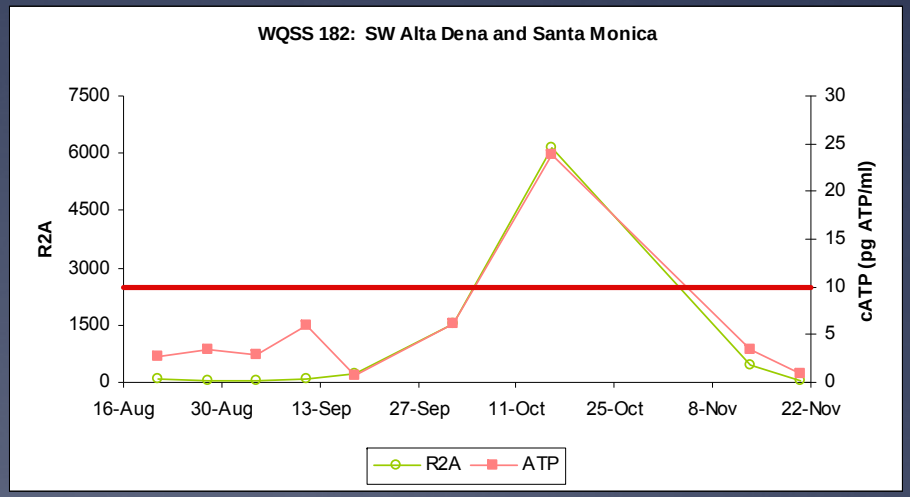
Luminultra – Results Interpretation

- The Luminultra test provides guidelines for interpretation of results. For potable water these include:
  - < 0.5 cATP (pg/mL) indicates good water quality
  - 0.5 to 10 cATP (pg/mL) indicates a potential problem may exist
  - > 10 cATP (pg/mL) indicates the need for corrective action

Luminultra Results

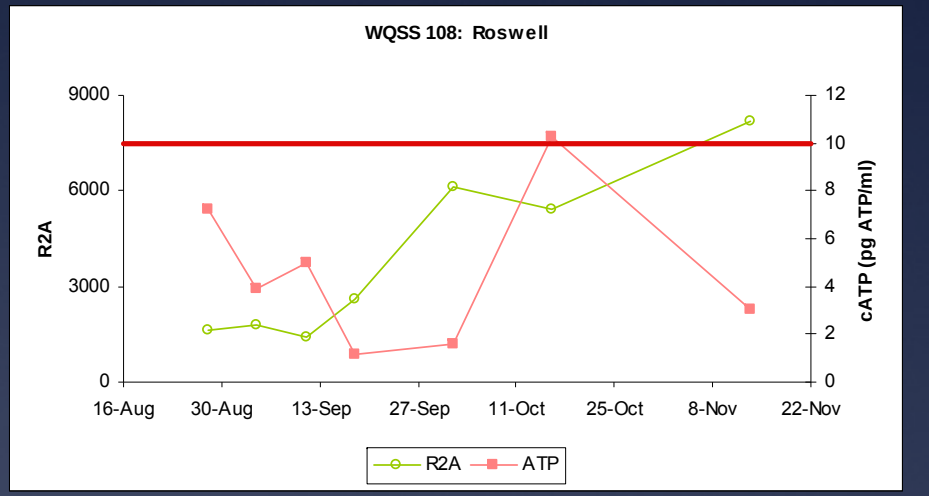
- The Luminultra test tracked water quality in the system
- R^2=0.55 for ATP vs R2A indicated a good relationship between the two tests

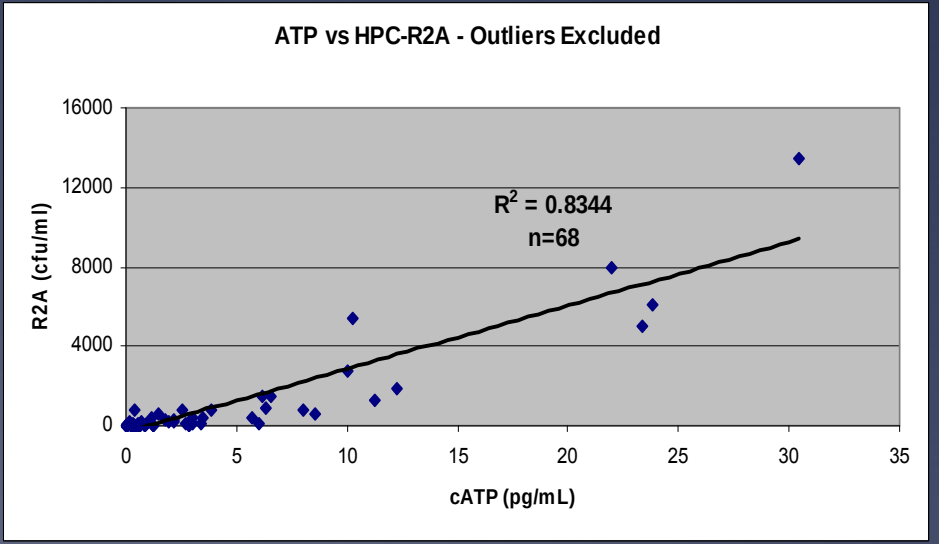




Luminultra Results Continued

- At a few sites, there appeared to be some interference in the ATP test
  - ATP results were low
  - But all other water quality parameters indicated high levels of microbial action (very high levels of R2A, low levels of chlorine, and varying levels of nitrite and nitrate)
- There were other sites with elevated nitrification rates where this phenomenon was not observed
- Removed these outliers from the analysis
  - R^2 of 0.83 for ATP and R2A
- Unclear at this point what caused this interference





Duplicates and Control

- Both tests gave very low results for the control (Nanopure water)
- To evaluate whether results from the tests were repeatable, we also collected 5 duplicates for each test – performed paired t-tests to evaluate the results for each assay
  - 3M: P = 0.78 for total, P=0.96 for free
  - Luminultra: P=0.97
- Conclusion = there was no significant difference between the duplicates for each analysis

Considerations for the QGA Assay

- Luminase enzyme is very temperature sensitive
- Test is light sensitive
- As with most tests, proper technique is essential
  - Pippetting
  - Dropper bottle (contains the standard) can provide different sized drops which can throw off the standardization
- Inhibitors – real or just us?

BONUS! Case Study Using ATP During the Total Coliform Incident in SW Portland - September 2013

- On September 18th, a routine TCR sample came back positive for TC, negative for EC
- Three resamples were collected at the site within 24 hours
- Repeat samples came back positive
- Ended up in a Tier 2 Violation

PORTLAND WATER BUREAU
FROM FOREST TO FAUCET

Nick Fish, Commissioner
David G. Shaff, Administrator

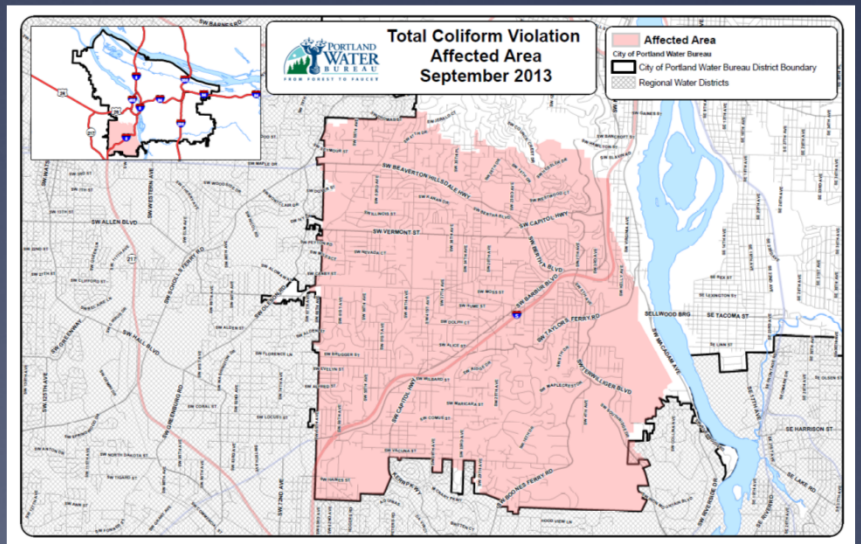
1120 SW 5th Avenue, Room 600
Portland, Oregon 97204-1926
Information: 503-823-7404
www.portlandoregon.gov/water

IMPORTANT INFORMATION ABOUT YOUR DRINKING WATER
Tests Detected Coliform Bacteria in Portland Water Bureau Drinking Water in SW Portland

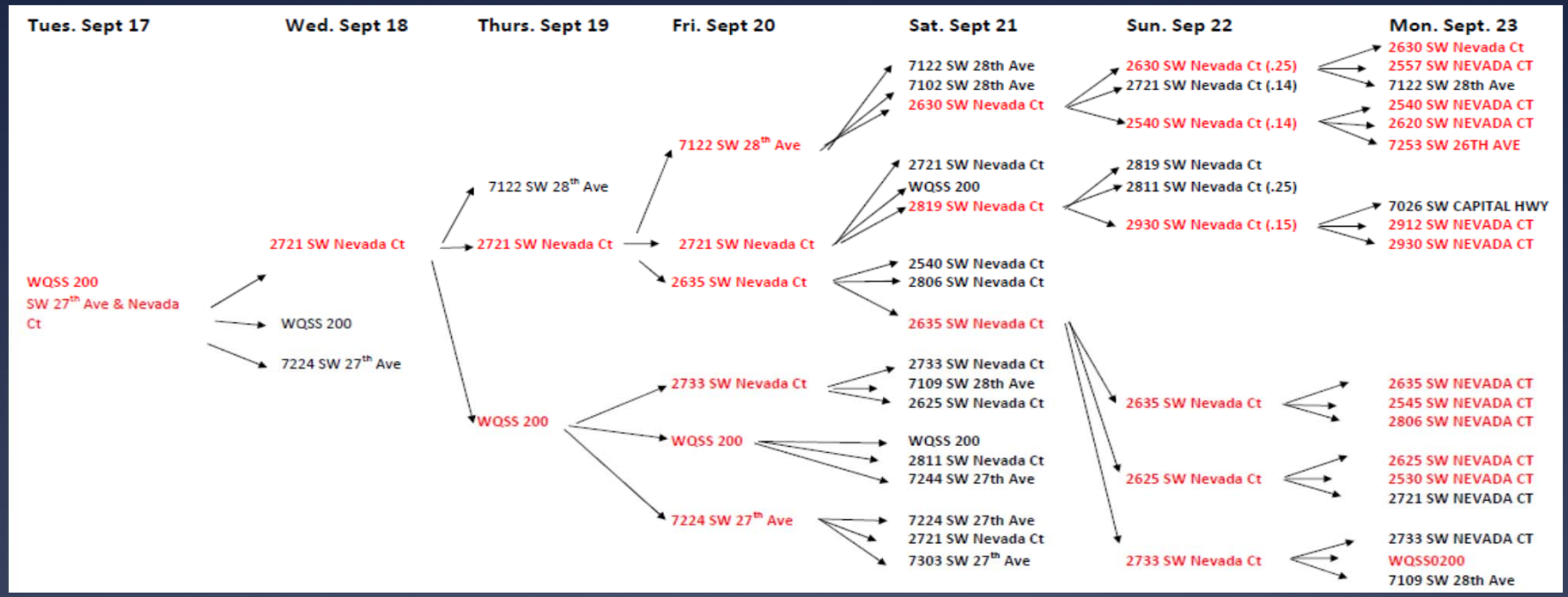
This information is being sent to all households and businesses that are in the area we believe may have been affected by the detection of bacteria in drinking water.

Our water system recently exceeded the drinking water standard for total coliforms. Although this incident was not an emergency, as our customers, we believe you have a right to know what happened, what you should do, and what we did to correct this situation. The language in this notice is required by drinking water regulations. For more information on this issue, please visit www.portlandoregon.gov/water/TCnotice.

The Portland Water Bureau routinely monitors for drinking water contaminants. During September, the Water Bureau took 384 samples to test for the presence of coliform bacteria. Forty-five (12%) of our samples showed the presence of total coliform bacteria. The standard is that no more than 5% of samples per month may do so.



Coliform Results in the Bertha Area



Positive coliform samples in red

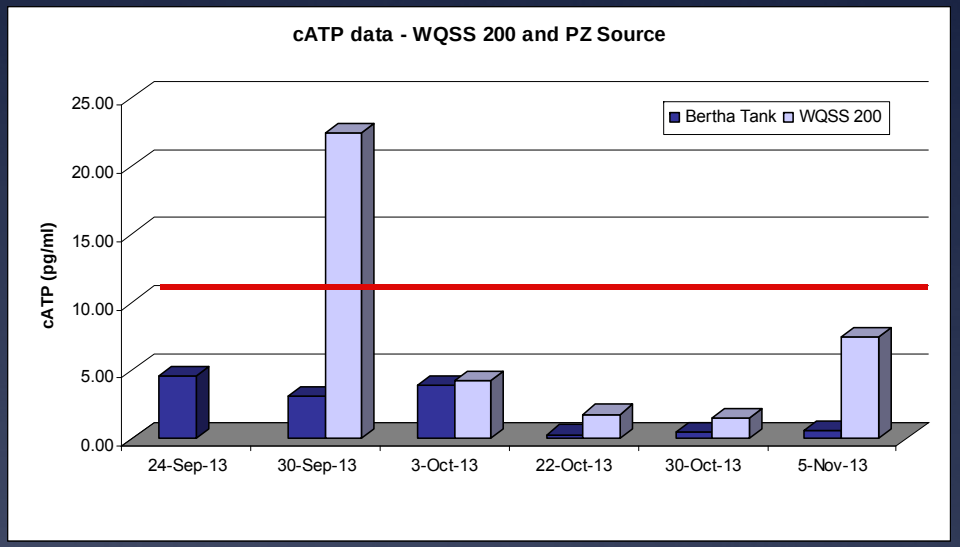
- Clearly we had a problem

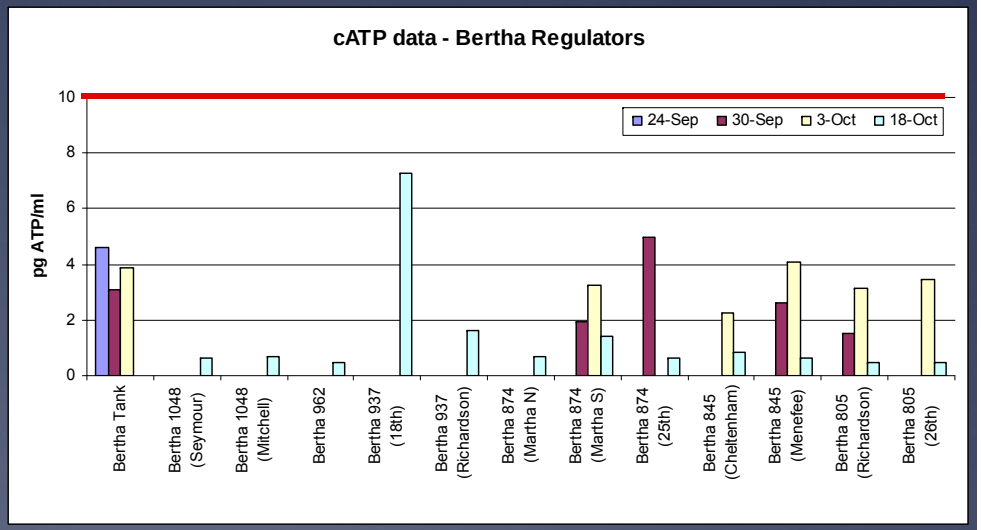
Increased Monitoring

- Increased water quality monitoring to determine the extent of the issue
  - Coliform surveillance sampling throughout entire system
  - Collected an additional 60+ coliform samples in our system in addition to our routine TCR samples
  - These results led us to conclude that the contamination was isolated to the Bertha area

ATP Analysis

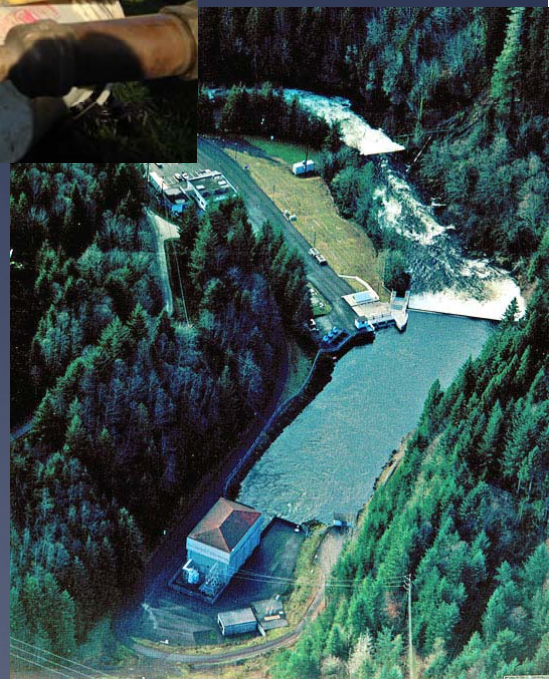
- Essential to figure out what was going on and where the problem was starting in the Bertha PZ
- Monitored chlorine and temperature but decided to throw ATP in the mix
  - Coliform testing could have thrown us back into the resampling loop
  - Did not want to wait 7 days for the R2A results



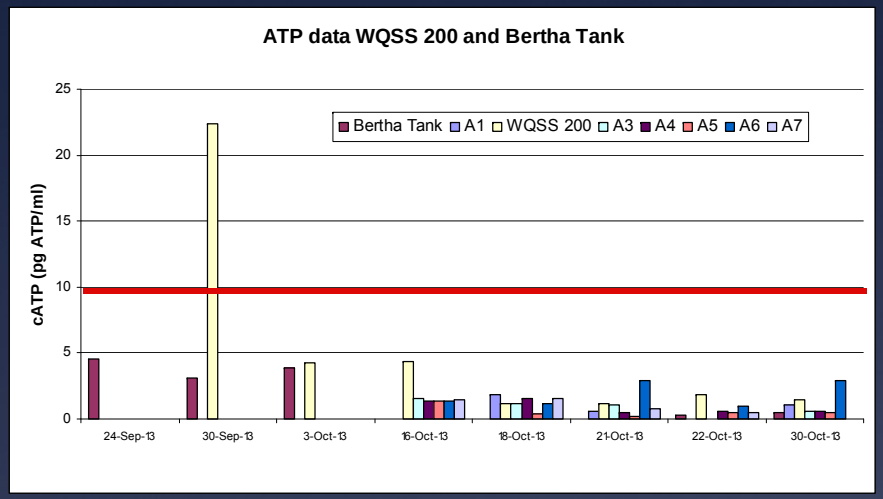


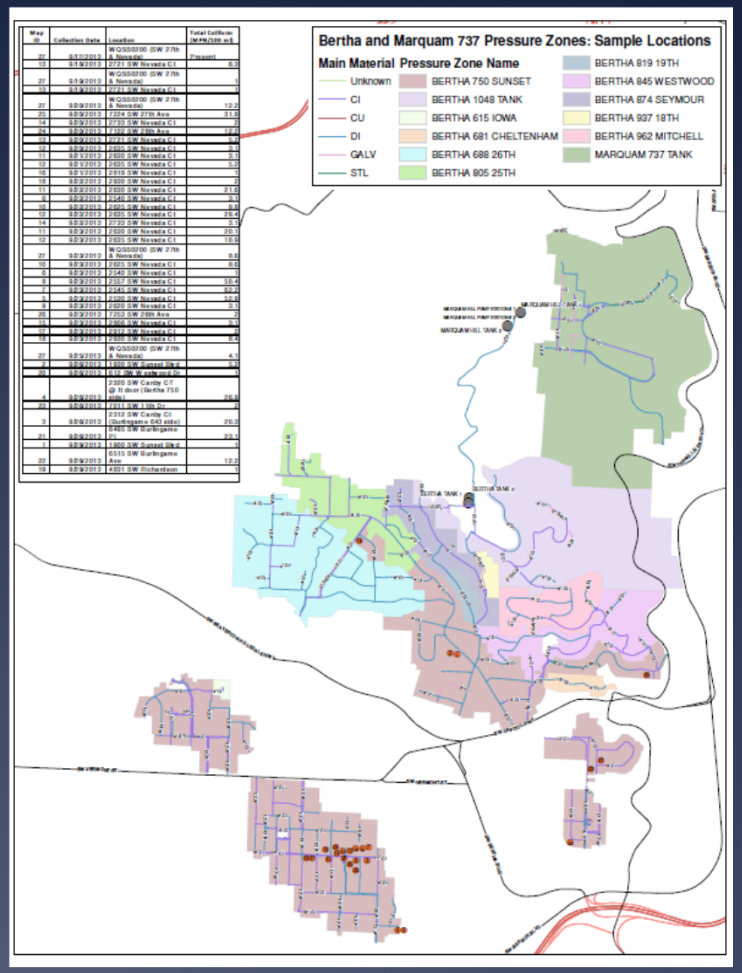
Mitigations

- Tried our routine mitigations (took storage out of service, adjusted pumping ops, lowered reservoir levels, spot flushed)
- When these did not solve the problem, took more aggressive steps
- UDF
- Raised the chloramine target level from 1.8 mg/L to 3 mg/L



Bertha Monitoring Continued





- Once monitoring results indicated that the corrective actions had been effective, five bacteriological samples were collected in Bertha 750 PZ (including WQSS 200) on Oct 21
- All samples were negative for TC/EC
- We felt that in this situation, in conjunction with other parameters monitored, ATP gave us extra confidence

Conclusions and Next Steps for ATP at PWB

- ATP (by the QGA) was a good indicator of nitrification
  - Useful tool, especially when results are needed immediately
- However, not a silver bullet and still needed to be interpreted along with other parameters to provide a full understanding of the water quality puzzle
  - Not currently planning to replace any of our standard monitoring parameters
- First need to investigate further the interferences that were observed
  - Analysis error?
  - Inhibitors present in those samples? High levels of metals, etc?
- Evaluate ATP for other uses
  - Tank cleaning
  - Source water evaluation

Questions?

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