

Faculty and PostDoc, Medical, Biomedical and Health Science

Introduction

- ❖ Maintaining the quality of water in an animal facility is mandatory for both the welfare of research animals and scientific reliability of research data.
- ❖ Vivarium animals are supplied with water from an automatic watering system through a stainless steel pipeline system. This study evaluates the quality of drinking water fed to the animals at Laboratory Animal Research Center (LARC)
- ❖ In two years of water monitoring program we evaluated the total microbial load, objectionable waterborne pathogens and other organic molecules present in living cells as an energy source ATP (adenosine triphosphate).
- ❖ In this study, data from both the methods of Heterotrophic plate count and ATPase assay (adenosine triphosphate) were compared for correlation, the results showed that there is no correlation and both these biological assays are two different parameters.

Aims

- ❖ To screen the animal drinking water for the objectionable Pathogenic microbes
- ❖ To check the integrity of the water supplying pipeline system in the animal house
- ❖ To check the efficacy of the Plate culturing method and ATP detection of biomolecules

Methods

Sampling: To evaluate the microbial load of animal drinking water, two sampling points from LARC vivarium were selected as per our environmental monitoring program

- ❖ Water from storage tank
- ❖ Water from IVC (Internally ventilated cages) Water valve

Total microbial count

Tryptic Soy agar is used for the enumeration of heterotrophic plate count. Water sample is dispensed into each of 2 sterile petri plates using sterile syringe. A molten agar cooled to 45°C is poured into the plates. The plates are incubated at 37 °C for 48 hours. The 48 hours period selected to give enough time for bacterial colonies to grow on agar surface. After 2 days of incubation the colonies are counted as an average in terms of CFU.

E.coli count

E.coli is considered as an indicator organism of fecal contamination of water. 10 ml of the sample is inoculated into transparent Fluid Lactose Medium using sterile syringe. The medium is incubated at 37 °C for 48 hours to check any sign of turbidity. In case of turbidity the sample will be streaked on differential and selective media for further confirmation. The characteristic green metallic sheen is the sign of positive sample.

Salmonella count

Salmonella is one of the leading water borne pathogen and can pose severe intestinal illness and other complications. A portion from Fluid Lactose Medium will be streaked onto the surface of Xylose lysine deoxy cholate medium and incubated in the agar medium at 30-35°C for 12-24 hours. Plates are checked for particular red colonies with or without black center.

Pseudomonas aeruginosa

Pseudomonads are highly versatile and has the ability to grow in distilled water and also in fresh water. Although Pseudomonas aeruginosa is considered as low significance but it can colonize in animals and cause illness (Pseudomoniasis).

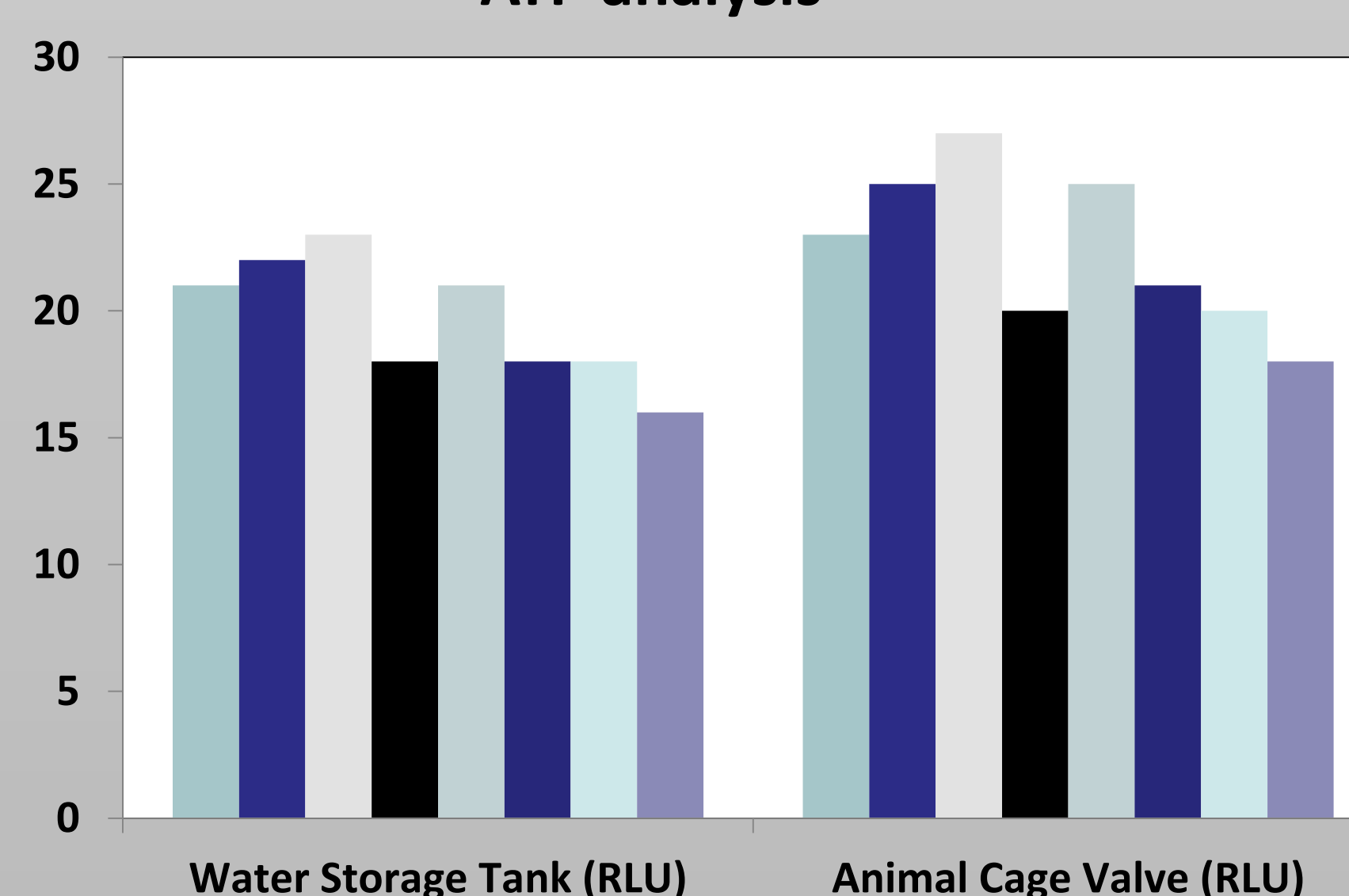
ATP Detection Assay

Water sample of 20 ml was collected in sterile water bottles from both points. 3M clean trace ATP swabs were used to dip into the sample for 5 seconds and reinserted into the swab tube. After the activation of ATP Luminometer, gives the reading in RLU

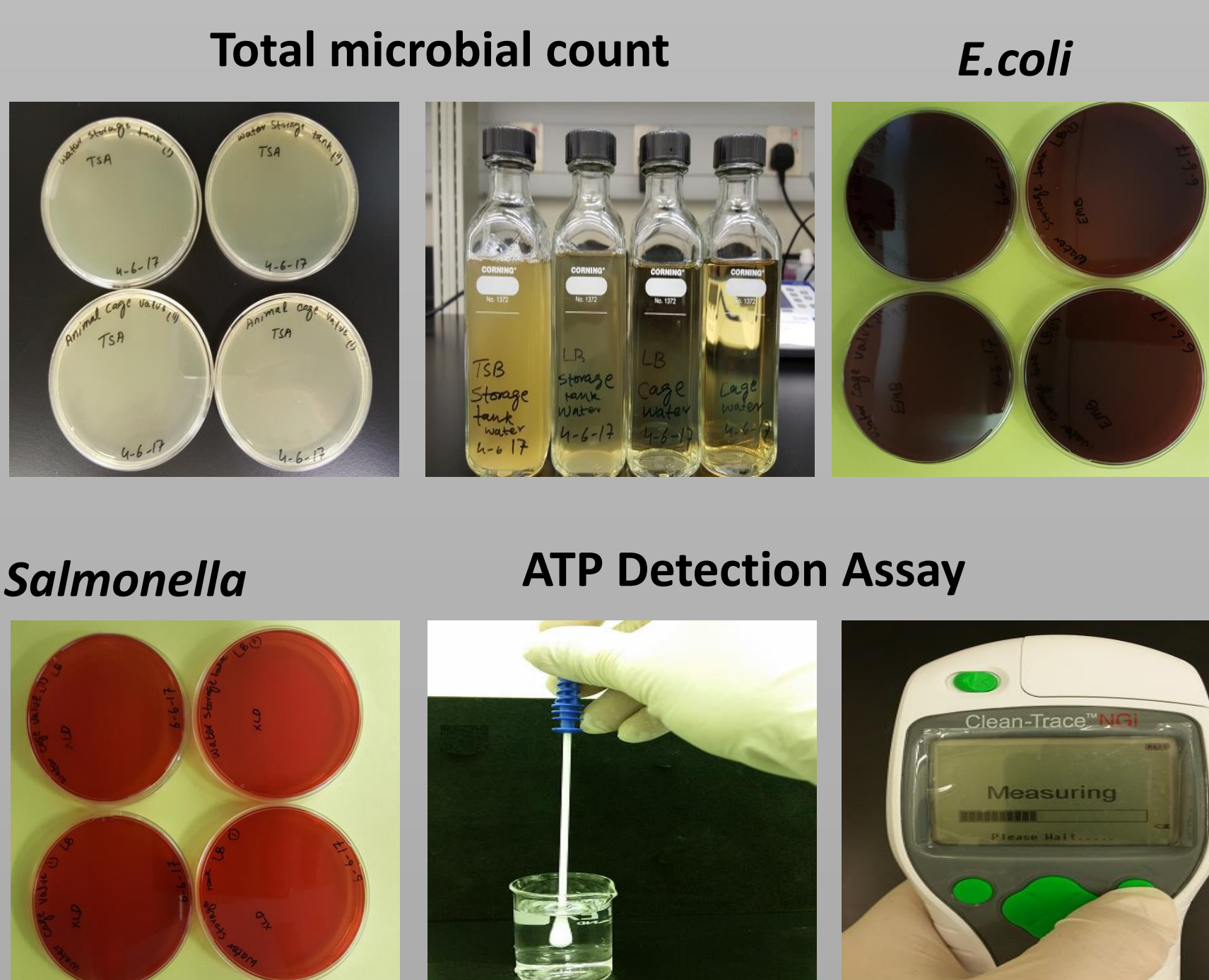
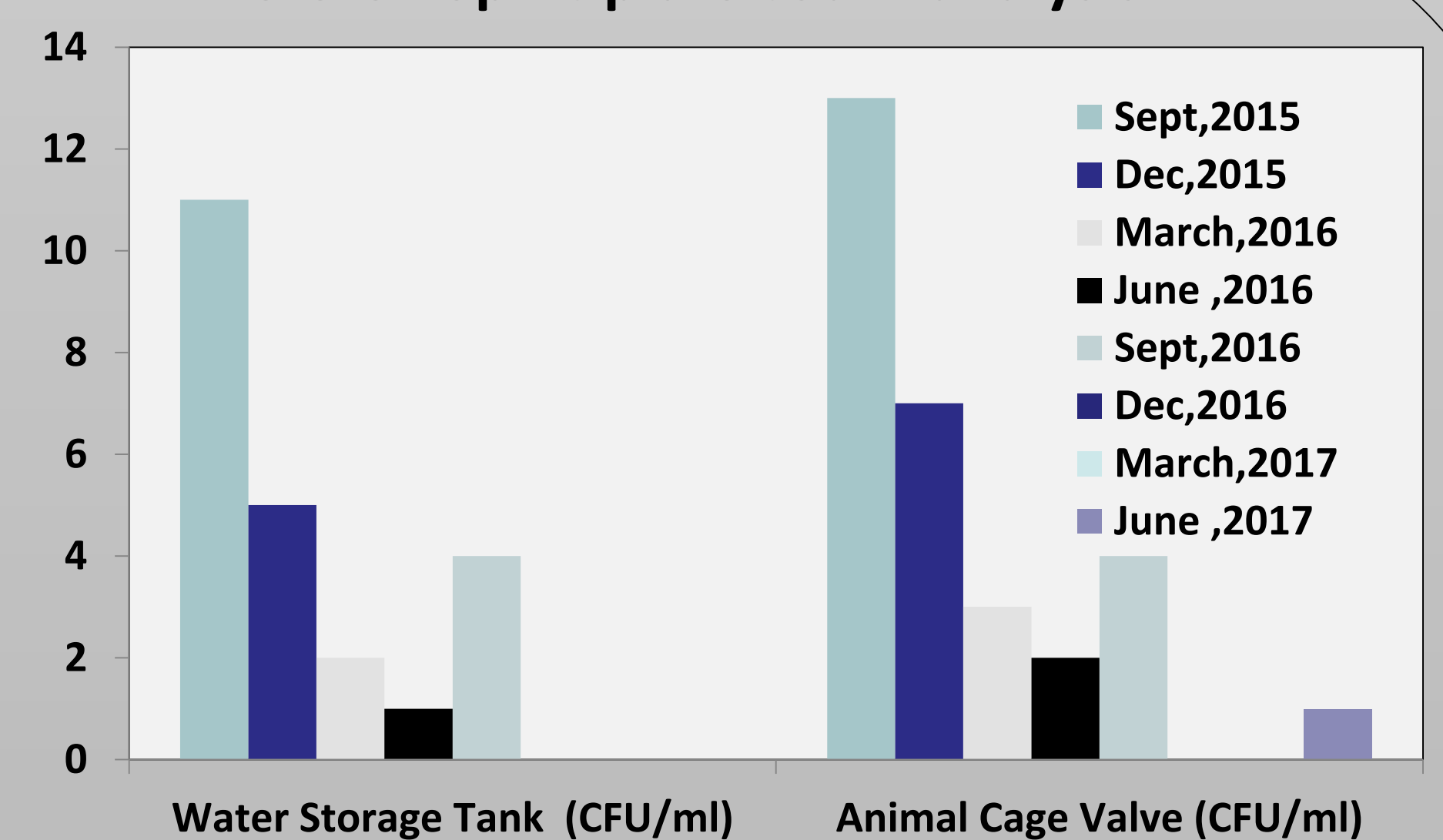
Results

Drinking water quality standard	
Microbial contaminants	Acceptable range
Heterotrophic plate count	< 100 Cfu/ml
Total coliform count	Zero Cfu/ml
Pseudomonas spp.	< 01 Cfu/ml
ATP detection Assay	< 31 RLU

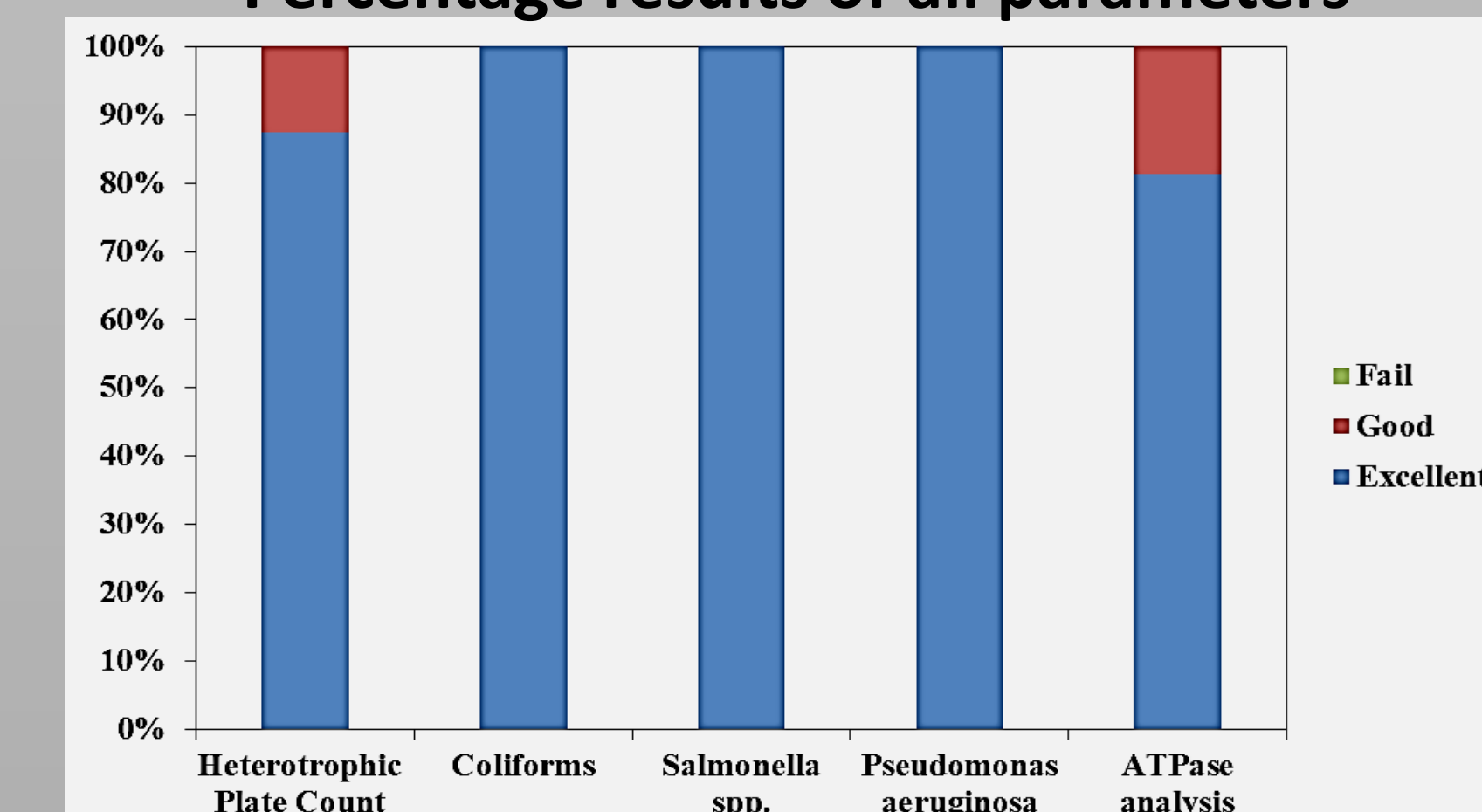
ATP analysis



Heterotrophic plate count analysis



Percentage results of all parameters



ATP Results of water sources

Month/year	Water Storage Tank (10x10 cm ²)	Animal cage valve (10x10 cm ²)
Sept, 2015	21 RLU	23 RLU
Dec, 2015	22 RLU	25 RLU
March, 2016	23 RLU	27 RLU
June, 2016	18 RLU	20 RLU
Sept, 2016	21 RLU	25 RLU
Dec, 2016	18 RLU	21 RLU
March, 2017	18 RLU	20 RLU
June, 2017	16 RLU	18 RLU

Comparison of culture technique and ATP detection assay

There is no correlation observed between ATP assay and heterotrophic plate count method. ATP is the total measurement all the biological contaminants in sample but culture technique is the culturing of specific objectionable microorganisms. These two parameters are different and have no effect on each other.

Conclusion

Edstrom watering system was newly installed and all the accessories were used for limited run, but gradually the system runs continuously and this improved the water quality. Moreover our microbial count of water samples are within the specific range and has negligible effect on health of research animals. The results show that the water meets the international standards and safe for animal use.