



# Monitoring of Microbial Pools Water Pollution Using Bioluminescence Assay

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**Abstract:** Microbial water pollution was investigated in pools lied in Baghdad city, which included (AL-Bayaa, AL-Jihad, AL-Yarmouk and AL-Raffedein). Swimming pools water was monitored using Bioluminescence assay which depends on the change of ATP amount that emitted from bacterial cells in the medium. Samples were analysed using two methods; first with traditional method (Heterotrophic Plate Count ) (HPC), second with Bioluminescence assay which featured by rapid and high accuracy. Also this assay does not need to an incubation period as in traditional method. Samples collected from pools were monitored by analyzing them during the period from the first day to the third day in which water has been renewed again after had been used by swimmers. Results showed continuous excess in the ATP amount through monitoring period and a strong relation between amounts of ATP and the number of growth colonies in the plates, the correlation coefficient was ( $R^2=0.9$ ).

**Key words:** Microbial, pools, Bioluminescence.

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## Introduction

ATP is essential for every metabolically active microbial cell, it is the main energy source for cellular functions such as enzymatic reactions (1). An ATP bioluminescence method is based on the ATP content of the bacteria in the water. The principle of this assay is the quantification of adenosine triphosphate, which is an energy-rich molecule found in all living cells. The bioluminescence is associated with the reaction between luciferase (enzyme), luciferin (substrate), and ATP (2). The light emitted during the reaction can be measured quantitatively and correlated with the ATP quantity

extracted from bacteria. Luciferase reactions are rapid, requiring only minutes to complete. There is no need to isolate and incubate bacteria before analyses. Luciferases from organisms that yield very bright bioluminescence have been adapted for use as reporters in high-throughput screening (HTS) assays, the most common being from the jellyfish *Aequorea Victoria*, the sea pansy *Renilla reniformis*, and the firefly *Photinus pyralis*. Firefly luciferase enables a variety of HTS applications, such as reporters for gene expression in cellular signaling assays, detection of the ATP found in cells as a measure of cell viability. (3)

Monitoring is a critical element in observing of water quality. It serves as a mean to determine how well source-to-tap barriers are functioning through provision of information about changes in water quality (4). This includes evaluating performance of treatment systems to remove and/or inactivate microorganisms, and testing water at the consumer's tap to indicate whether microorganisms of human health significance might be present. From a public health perspective, monitoring is a means to identify emerging water quality issues that can in turn be used to implement response measures to minimize adverse public health outcomes from consumption of unsafe drinking water (5). In support of this important role, many developed nations have implemented legislation that requires monitoring of a suite of human health relevant chemical and microbiological parameters to protect public health. The aim of this study was to install a rapid and sensitive assay to detect and monitor pools water pollution.

### **Materials and Methods**

Illuminometer (GloMax) which had capable of reading multiwell plates and BacTiter-Glo™ Microbial kit was used for this purpose. Values of Relative Light Unit (RLU) which showed the result of bioluminescent system analyses were obtained using opaque-walled multiwell plates, multichannel pipette, or automated pipetting station for delivering reagent, plate shaker or other device for mixing the contents of multiwell plates. Nutrient agar was used for traditional method (HPC), while BacTiter-Glo™ Microbial kit was used for Bioluminescence assay.

### **Sampling**

Water samples were collected from several swimming pools, in Baghdad city (AL-Bayaa, AL-Yarmulke, AL-Raffedein and AL-Jihad) in order to monitor changes in the amount of ATP from the first day on which the water recycling to third day. One sample in five different periods (0-10-30-75 hrs.) were taken from each swimming pool.

### **Examination of Water Samples with HPC Method and Bioluminescence Assay**

Samples were analyzed directory at a time were taken from pools. Each sample was tested with both Heterotrophic Plate Count (HPC) and Bioluminescence assay immediately at 0 times , after 10 hours, after 30 hours then after 50 hours and finally after 72 hours in order to monitor the amount of ATP over time .

### **Media Preparation**

Nutrient agar was prepared according to the manufacturing company instructions. They were brought to boil in water bath to dissolve all constituents completely, and then sterilized by autoclaving at 121°C for 15 minutes at 15 pounds per square inch, and then the media were incubated at 37° C for 24 hours to ensure sterility (6).

### **Conventional Methods**

The Heterotrophic Plate Count (HPC) was used to estimate the number of live heterotrophic bacteria present in a water sample. (1) ml of each pool samples (AL -Bayaa, AL- AL-Jihad, AL-Yarmouk and AL-Raffedein) were

inoculated onto Nutrient agar plates at 28 °C for 48 hours. The HPC results were reported as Colony Forming Units per milliliter CFU/ml (7).

### Bioluminescence Assay

In this assay Glomax (illuminator) (Figure 1), which was used with BacTiter-Glo™ Microbial kit and

multiwell-plate (96 wells). The assay procedure involves adding a single reagent (BacTiter-Glo™ Reagent) directly to bacterial cells in medium and measuring luminescence (8). The luminescent signal (RLU) was proportional to the amount of ATP present, which in turn proportional to the number of cells in culture (9).



Figure (1) The GloMax®-Multi+ Detection luminometers System. (Alice *et al.*, 2008)

### Bioluminescent Reagent

All the used reagents were prepared according to the manufactures instruction of the kit.

### Bioluminescence Assay Steps

- 1-An opaque-walled multiwell plate 96-well plate was used.
2. 100µl of BacTiter-Glo™ Reagent was added to 100µl of water sample present in each well.
3. Control was prepared by adding 200µl of (BacTiter-Glo kit) to plate well.

4. The contents were mixed briefly on an orbital shaker and incubated for five minutes.
5. Glomax (illuminator) was used to get luminescent data.
6. Luminescence data (RLU) was recorded. (10)

Note: All steps were performed at room temperature (22–25°C).

### Results and Discussion

#### Monitoring Results

To prove the applicability of the ATP bioluminescence method, the data

obtained from the ATP bioluminescence and HPC methods were compared.

The BacTiter-Glo™ kit generates a “glow-type” luminescent signal (RLU), produced by the luciferase reaction, which has a signal half-life generally over 30 minutes depending on the bacterium and medium (10). The assay has been shown to detect a variety of bacteria, yeast, and fungi (11).

Data which were collected rapidly from swimming pools in Baghdad city (AL-Bayaa, AL-Yarmulke, AL-Raffedein and AL-Jihad) by bioluminescence assay was matched to the result of conventional method (Heterotrophic Plate Count HPC) as shown in table (1 and 2), the difference between the two methods was the time factor. The data of bioluminescence assay was obtained

through 4 minutes. While the data of conventional method were needed at least two days to be obtained, at that time water was consumed by swimmers. It was noticed that the number of bacteria and amount of ATP (RLU) at zero time in AL-Yarmouk pool was less than that in other pools.

Pools water after 72 hours showed high dangerous levels of bacteria and high amount of ATP, the number of bacteria more than 30 CFU/ml that made water unusable (12). The benefit of new method was rapidly detect of bacteria growth which help in avoiding such as dangerous level of bacteria.

The disadvantage of this method isn't diagnosis the type of bacteria which needs to Immunomagnetic separation (IMS) protocol (13).

**Table (1) Number of bacterial colonies in the pools water samples in different period**

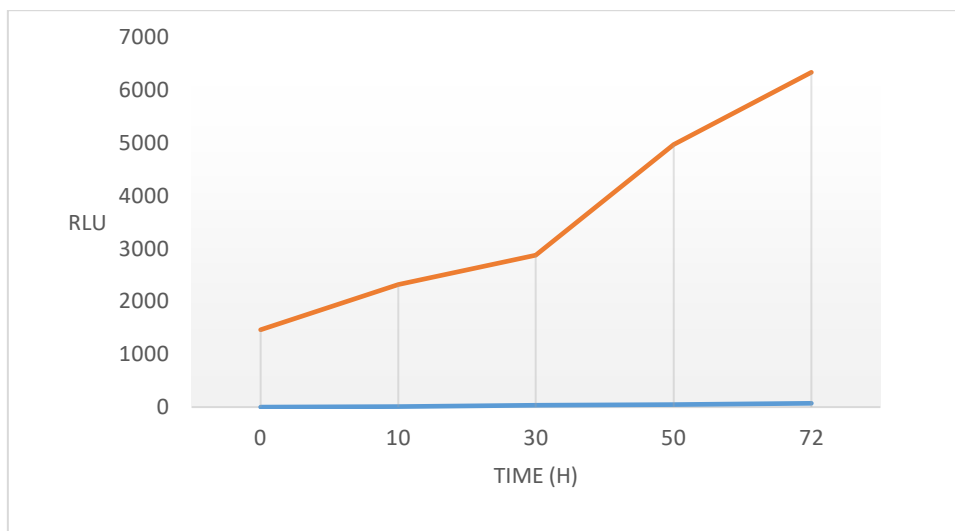
Pools areas	Colony forming unit (CFU)				
	zero time	After 10 hours	After 30 hours	After 50 hours	After 72 hours
AL-Bayaa	2	14	19	29	45
AL-Jihad	3	10	20	30	51
AL-Raffedein	2	22	25	35	53
AL-Yarmulke	1	11	21	27	48

**Table (2) Amount of ATP (RLU) in the pools water samples in different period**

Pools areas	Relative Light Unit(RLU)				
	zero time	After 10 hours	After 30 hours	After 50 hours	After 72 hours
AL-Bayaa	1464.100	2322.349	2875.710	4965.754	6337.890
AL-Jihad	1666.130	1820.120	3280.666	5422.170	7084.360
AL-Raffedein	1588	2801.300	3860.130	5688.430	7154.410
AL-Yarmuke	1390	1901.670	2408.340	4790.401	6698.110

The relation of RLU for water samples was correlated by plotting values of RLU on the x-axis and times readings on the y-axis. It was noticed that the

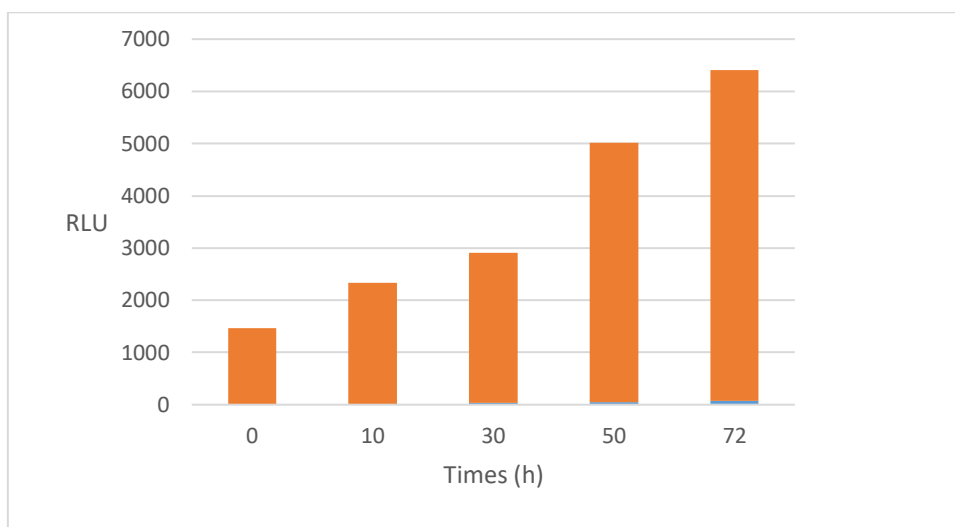
amount of ATP was increasing with time as showed in (Figures 3 and 4).



**Figure (2) Amount of ATP with time through 3days of AL-Jihad swimming pools**

The risk of illness or infection has been linked to contamination of the pool water. So to avoid that risk, monitoring with repaying method such as

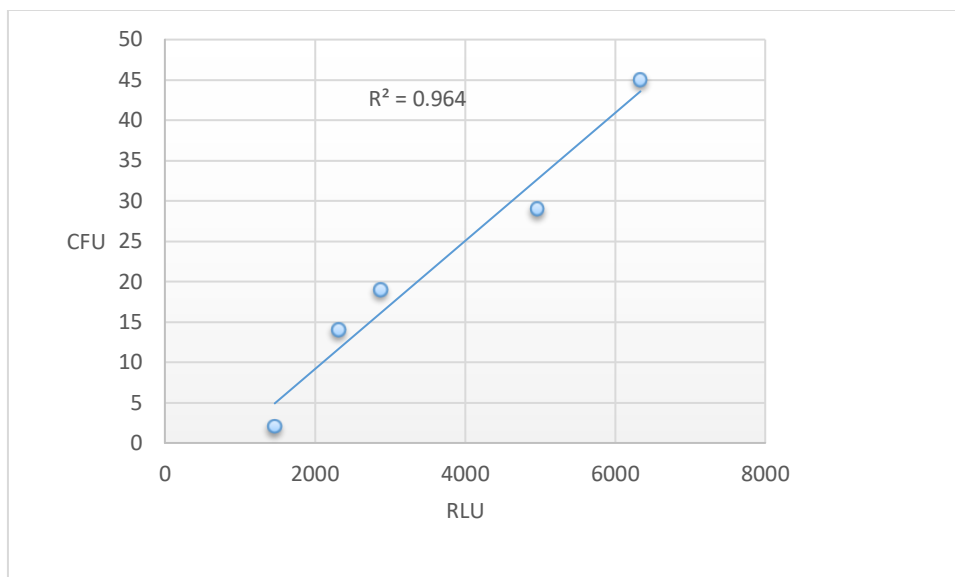
bioluminescence assay considered as an early sense of water bodies to remedy the various risks arising from increasing of biological pollutions.



**Figure (3) Amount of ATP for AL-Bayaa pool water samples with time**

The correlation coefficient between number of colonies on the nutrient agar (CFU) and RLU was found to be ( $R^2=0.964$ ).

9641), this indicate the ability of this method to detect the amount of bacteria in a short time as showed in figure (4).



**Figure (4) Correlation between number of bacteria CFU and amount of ATP (RLU) in AL-Yarmulke pool water**

Monitoring data which were obtained from bioluminescence assay of pools in Baghdad city (AL-Bayaa, AL-Yarmulke, AL-Raffedein and AL-Jihad) can be conducted for many purposes. Characterize waters and identify changes or trends in water quality over time; identify specific existing or emerging water quality problems; gather information to design specific pollution prevention or remediation programs and determine whether program goals, such as compliance with pollution regulations or implementation of effective pollution control actions.

### Conclusion

The ATP bioluminescence assays provide a rapid means of enumerating

total numbers of viable bacterial cells. The estimation of the heterotrophic plate count can be done in minutes a strong correlation exists between HPC and ATP assay. Using bioluminescence allowing the prediction of the HPC from the ATP test due to the small size of the illuminometer and the high sensitivity of the assay can also be used as a tool to monitor the water pollution specially the drinking water because its effect on human healthy.

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