Early-AOC Test Kit

Applications Guide

Version 1.2

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1. introduction

1.1 Technology overview - determination of AOC (Assimilable Organic Carbon) in water

Regrowth potential of heterotrophic bacteria in potable water depends mainly on the presence of assimilable organic carbon source. Many bacteria are capable of dividing in water containing as low as 2-5 ppb of different carbon sources. The potential hazard in the distribution of pathogenic bacteria and the effect of bacterial growth on water quality and biofilm formation demands the addition of proper biocidal agents to water supplies. Since such treatment is costly and may result in the formation of toxic derivatives, determination of the regrowth potential of bacteria in water is vital.
Not all organic compounds present in water support microbial growth. Hence, it is important to be able to quantitatively measure the levels of biodegradable (or assimilable, utilizable) organic matter. Various chemical parameters such as total or dissolved organic carbon (TOC, DOC) proved inadequate for this purpose; it has been shown (Van der Kooij et al, 1982; Werner and Hambsch, 1986,1988) that the fraction of the total organic carbon pool which is available for biodegradation can be very small and is generally highly variable.

1.2 What is unique in the Early-AOC Test?

The standard procedures for AOC determination in water rely on direct measurement of microbial growth; either a single- or multiple-species inocula, as well as a natural consortium of indigenous microbial flora have been used in different assays (Hambsch and Werner, 1989; APHA, 1996; Rice et al, 1990). While generally accurate and reliable, these technologies are tedious and require days to weeks before data are available; obviously, results obtained after such a long procedure have little value.
A suitable test for AOC in water should be rapid, inexpensive, and sensitive enough to detect very low concentrations of diverse groups of utilizable organic compounds in water. CheckLight's AOC test stands in the above requirements. The test is very simple, short, and shows high correlation with the capacity of bacteria to divide in the studied water.

1.2.1 The principle of the Early-AOC test

The test is based on the effect of assimilable organic compounds on the development of luminescence in Vibrio harveyi. The bacteria are given all the environmental and nutritional conditions necessary for light production, except an organic carbon source, instead of which the cells are exposed to the tested sample. The luminous bacteria are provided in a freeze-dried state. Upon hydration in the water sample, these bacteria undergo prompt induction of the luminescence system if the sample contains assimilable organic compounds. Luminescence increases with time, with an intensity dependent on the concentration of the organic compound. Sub-ppm concentrations of different kinds of assimilable organic compounds can be determined within 2-3 hours.

1.2.2 Dechlorination of water samples

The presence of chlorine and its byproducts lead to rapid decay of bacterial bioluminescence. Sodium thiosulfate is included in the assay buffer to chelate up to 2 ppm chlorine. Higher concentrations of chlorine should be properly diluted in clean water prior to addition of thiosulphate (up to 100ppm) , or removed by other means.

1.2.3 How might toxicants present in the tested water sample effect the results?

Whenever a water sample contains both assimilable organic compounds (that promote light development) and toxic compounds (that might diminish light level), the luminescence obtained may not behave in a concentration-dependent manner. Spiking various toxicants (tested at concentrations that were a hundred fold higher than the allowable level in drinking water) into water with 10-1000 ppb carbon source, did not affect the test performance.

It can not be ruled out, however, that low AOC water samples containing a high concentration of toxicants might exhibit inhibited luminescence. When the AOC level is high enough in the toxic sample, luminescence will develop in diluted water samples, but not in the concentrated ones.

One way of gauging the possible presence of toxic elements in the tested water sample is to spike the tested sample with increasing concentrations of the Carbon Cocktail Solution provided.

1.2.4 Repeat Dispensing in the Early-AOC Test: Recommended Products

All of CheckLight's **lab test procedures** include the dispensing of very low volumes (10 microliters) of hydrated bacterial suspension into the assay mix.

Since the overall time span of each test is short and light emitted by the bacteria in the sample is compared to light emitted in the negative control, one has to ensure rapid dispensing. Moreover, as each 10 microliter aliquot holds about one million cells, dispensing 9 or 11 microliter, leads to a dramatic change in emitted light, and hence, to skewed results. It is therefore essential to ensure accurate dispensing.

Given the above, the use of an automatic pipettor or repeat dispenser provides the optimal solution and should be regarded as an essential tool.

There are numerous products on the market. Among the recommended options for highly reliable products are:

1. Finnpipette Stepper model 4540 from Thermo Scientific-
http://www.thermo.com/com/cda/product/detail/0,1055,19353,00.html

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Highlighting Water Safety

2. Ripette electronic pippetor from Ritter -
http://www.ritter-online.de/e/medical_care/ripette/index.php
A less sophisticated line of products is the syringe-less version.

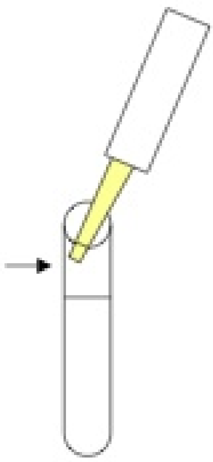
We provide a product manufactured by Microlit -
http://www.microlit.com/elec.htm

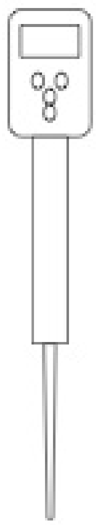
Instructions for use are provided in the next page.

A short training video clip on pipetting is also avaliable for download on our web site.

1.2.4.1 Instructions For Operating The Electronic Micropipette (Repeat Dispenser)

1. The device has 3 operation modes to choose from. The one relevant for use with CheckLight's kits is **CASE III – Stepper Mode**.
2. Follow the instructions for setting the Stepper Mode in the provided Operation Manual.
3. During the dispensing phase it is very important to **touch the inner side of the tube with the edge of the dispensing tip** in order to ensure that each drop is released and captured in the tube.





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2. Application Of Early-AOC In Drinking Water Treatment

2.1 Overview

2.1.1 The Present Situation

Water utilities are faced with the dilemma of having to balance between escalating demands to reduce the level of disinfection by-products (DBPs) in their drinking water supply, but at the same time they also have to ensure that the presence of microorganisms in it is severely restricted.

Acceptable methods for determining the causes of bacterial re-growth and prediction of their presence in water distribution systems have typically involved collecting data from the field and then statistically interpreting the results. However, cost factors usually mean that this approach is limited to low frequency of sampling and limited number of sampling sites.

Testing for chemical parameters only, such as total or dissolved organic carbon (TOC, DOC) prove inadequate for monitoring bacterial re-growth potential because it has been shown that the fraction of the total carbon pool available in water for biodegradation can be very small and is generally highly variable.

Ironically, the one test that is acceptable, AOC, is in itself problematic because the methodology involves a long delay time before results can be obtained. The test involves inoculating the collected water samples with a bacterial inoculum (intrinsic population or a standard culture). Only after a period varying between 5 and 30 days can the increase in viable counts and/or the decrease in DO, be determined.
Obviously, results obtained after such a long time are only valuable as historical information for the records but have little practical value for the authority in its effort to control the quality of drinking water.

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As a result of this unsatisfactory situation, the authorities usually have to guess the real state of the water and either over or under disinfect it with the consequent waste of money or breach of fiscal quality standards.

2.1.2 The Problem

Growth of bacteria in drinking water distribution and storage systems can lead to proliferation of health hazards, to the development of unacceptable taste and odor and to water turbidity. The rate of re-growth of bacteria in water after the disinfection process is influenced mainly by the concentration of biodegradable organic carbon (BDOC) or assimilable organic carbon (AOC) present in the water. Both parameters represent the fraction of the dissolved organic carbon (DOC) that can be metabolized by bacteria in a given period of time. Urbanization creates storm water runoff from paved areas and the sequential use and return of waters to rivers and lakes increases the absolute mass of biodegradable organic matter present in source water. Urbanization also generates an increase in the water service area with the consequential greater complexity in operating the distribution system. This often results in an increase of water residence time in the system and a reduction in disinfectant residual at some locations. Poor disinfection increases the rate of bacterial re-growth in the distribution system.

2.2 Case Study - Real-Time Monitoring of AOC in Drinking Water

A water utility was looking for a measurement method of readily available organic matter that could provide real-time precise measurements so as to enable effective management of water treatment regime and achieve consistent water quality within budget.

CheckLight was able to demonstrate that its proprietary Early-AOC Test Kit could provide the means to measure in real-time the levels of readily available organic carbon in raw and finished drinking water sources. Acquiring this vital information would enable the water utility provider to take timely preventative action to avoid bacterial re-growth, optimally organize its disinfection program and reduce the presence of excess toxic DBPs in the water.

CheckLight's specially formulated reagent is exposed to serial dilutions of the sampled water. Final readings are obtained after only two hours of incubation at ambient temperature from measuring the biological luminescence that subsequently develops in the samples. The level of luminescence is proportional to the concentration of available organic carbon. Figure 1 shows the resultant measurements of the standard Carbon Cocktail used as reference and positive control in the test system. The correlation is straightforward: **the more luminescence, the higher the concentration of AOC.**

In order to verify the accuracy and reliability of the test, 26 samples were collected from different untreated water sources along the Israeli Water Carrier System. Samples were then tested using both CheckLight's Early-AOC Test and the Standard (van der Kooij)7 days test. Figure 2 shows the high correlation that was obtained from identical samples, between the standard AOC test (measured in ug acetate/L) and the Early-AOC Test (measured in ug Carbon Cocktail equivalent units/L).

Figure 1:

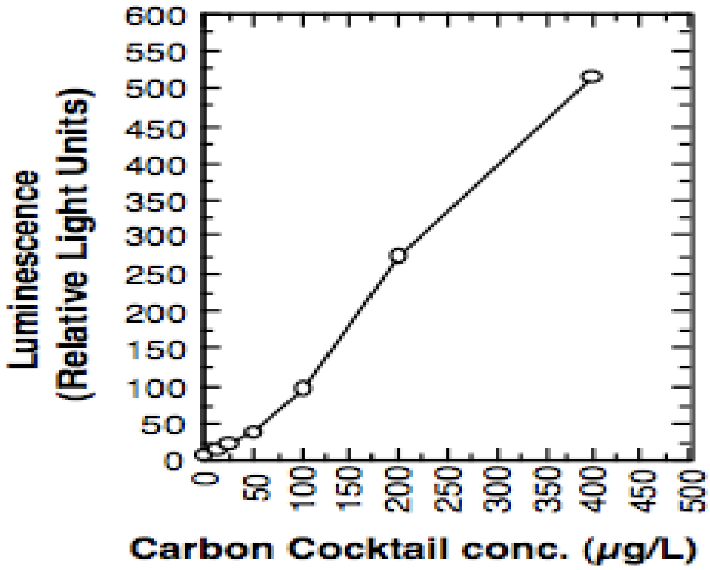

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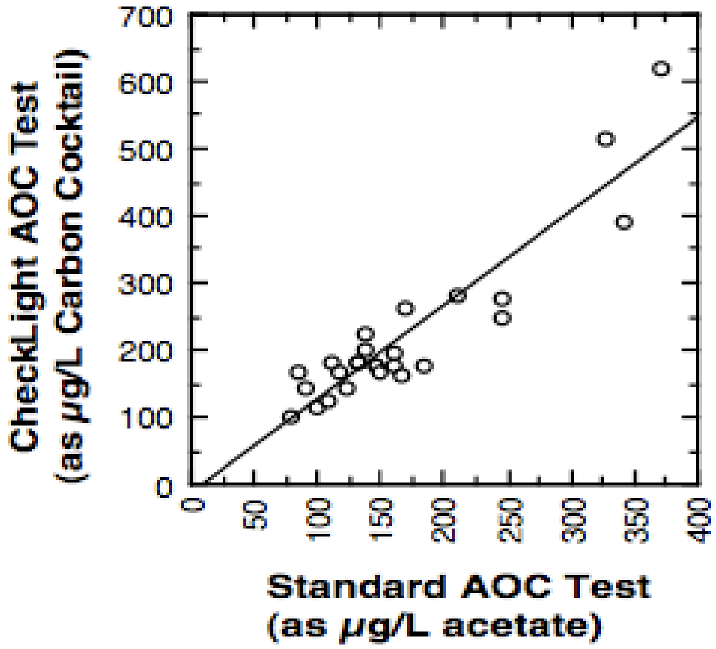
Figure 2:



A high correlation (r=0.864) was found between the results of the two testing methods.

The Outcome

The water utility operators were pleased with the results from the comparison and can now regularly use the Early-AOC test kit to measure the quality of their water and obtain timely information about its quality.

CheckLight kits are favoured by the authorities because of the following reasons:

Time saving - accurate and meaningful results of dozens of samples obtained within a few hours.
Easy to use – personnel do not require special laboratory skills to use kits
Reliable – data highly correlative with standard tests
Cost Effective - enables frequent monitoring for rapid response to changing conditions

The Early-AOC test kits are also being successfully used to monitor water at various pre/post-filtration steps desalination and purification processes, optimizing the timing of filter backwash, and providing real time detection of operation failure. A modified version of the test, **Early-AOC-DES** (utilizing a different natural luminescent bacterial strain) is available for monitoring **brackish and seawater.**

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3. Application Of Early-AOC In RO Desalination Plants

3.1 Overview

3.1.1 The Situation

One of the most difficult problems in water purification systems is microbiological contamination. Microbial growth in a water system not only reduces water quality, but if left unattended, it will eventually form biofilms on the membranes that can lead to more plant down-time, higher operating cost due to higher feed pressures, higher chemical cleaning costs, shorter membrane life, and degradation of permeate quality.
In light of the above, proper monitoring of each section of the RO plant is of vital importance to detect any biofilm formation at its early stage.

3.1.2 The Problem

A crucial parameter in biofouling development is the availability of nutrients that can support microbial growth in the system. The most common method used to measure its level is the TOC test (Total Organic Carbon). However, to obtain an accurate estimate of bacterial regrowth potential one has to determine the assimilable fraction of the total organic carbon. Most of the standard or proposed AOC methodologies are hindered by their long duration, cost, labour requirement, and lack of robustness. They involve the inoculation of the studied water sample with a bacterial inoculum (intrinsic population or a standard culture); after a time period varying between 5 and 30 days, the increase in viable counts and/or the decrease in DOC are determined. Obviously, results obtained after such a long procedure have little practical value.

3.2 Case Study - Utilizing Early-AOC Test To Monitor Biofouling Potential In Water Purification Systems

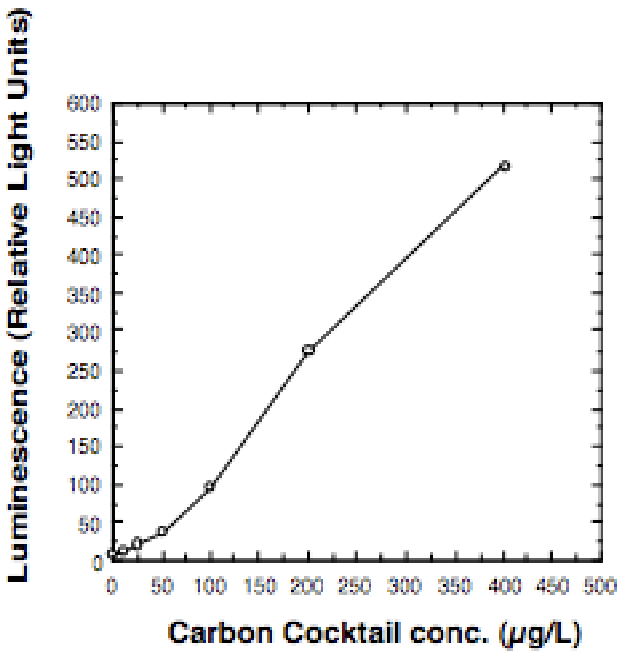
CheckLight was able to demonstrate that its Early-AOC Test Kit can provide vital real-time measurement of readily available organic carbon in water. Acquiring this information in real-time would enable water purification system operators to take timely preventative action to protect expensive filtration units from biofouling and biofilm formation.

The Method

The kit reagent is exposed to serial dilutions of the pasteurized water sample and developed luminescence is recorded after two hours of incubation at ambient temperature. The level of luminescence accurately reflects the concentration of bio-available organic carbon.

Figure 1, shows the resultant measurements of the standard Carbon Cocktail used as reference and positive control in the test system.

Figure 1-



Data analysis is straightforward: **the higher the luminescence, the higher the concentration of AOC.**

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Results

The results obtained from the analysis of two plants (A,B) with similar water purification setups are shown below. The main objective of this sampling and analysis technique is to be able to localize or isolate the source of any bioactivity before it starts to spread and affect other parts of the system, especially the RO membranes. Sampling points were chosen as to adequately cover the entire RO plant system and were subjected to Early-AOC Testing.

Table 1- Detected concentrations of AOC along the tretament train of two plants (presented in µg carbon equivalent units/L).

Sampling Point	Plant A	Plant B
Raw water intake	490	420
After sand filter	265	205
After carbon filters	390	77
After micron filters	375	69
RO product	<20	<20

As can be seen, the water purification cascade in Plant B operates well in terms of organic carbon removal. However, in Plant A, a problem was spotted in the granular activated carbon step. Not only did it fail to remove the AOC , but it also increased its level in the outflow, most probably due to microbial contamination inside or on the surface of the filter bed and the release of metabolism by-products and debri. Real-time detection of the failure point in the process, enabled the operators to undertake rapid corrective measures to protect the RO membranes from high AOC exposure that might have lead to biofilm induction.

Conclusions

CheckLight's Early-AOC Test Kit is a simple, reliable bioassay for real-time, low-cost determination of the presence of readily utilizable organic carbon in water. It can also be easily applied to monitor AOC in **brackish water and seawater desalination systems**.

Benefits:

- **Time saving** - accurate and meaningful results of dozens of samples can be obtained within 2-3 hours. No need for sample transportation to external labs.
- **Reliable** - data highly correlative with standard tests.
- **Easy to use** - no special laboratory skills required for operation.
- **Cost effective** - enabling frequent monitoring to rapidly detect dangerous changes in water quality.

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Applications:

The test enables QA/QC supervisors to:

- Monitor water at various pre- and post-filtration steps.
- Optimize timing of filter backwash.
- Provide very early warning of membrane biofouling.
- Reduce operation & maintenance costs (i.e.,lowest total cost of water production).

4. Frequently asked questions

Q: Are luminous bacteria dangerous? Do I need to be a trained microbiologist in order to be able to conduct CheckLight's assays?

A: Luminous bacteria are not pathogenic and are harmless. No special skill is required to carry out the different tests other than very basic laboratory techniques (pipetting, dilutions etc) and equipment (pipettor, tips, luminometer).

Q: What is the danger in having high nutrients level in drinking water?

A: High nutrient levels in drinking water may lead to the following drinking water problems:

• Increased levels of microbes, including opportunistic pathogens, in the bulk water, as well as in the pipe biofilm and sediments.

• Loss of disinfectant residual through reactions between disinfectant and nutrients.

• Production of toxic and/or carcinogenic DBPs through reactions between disinfectant and nutrients.

• Unreliability of total coliform sampling due to increased growth of heterotrophic bacteria, resulting in false-positives or false-negative coliform tests. Coliform sampling may also become unreliable due to stimulated growth on pipe biofilms and sediments. These increased numbers may not be represented in coliform samples of bulk drinking water.

• Development of aesthetic problems

Q: What are the benefits of obtaining rapid information on AOC levels?

A: Acquiring this vital information would enable the water utility provider to take timely preventative action to avoid bacterial re-growth, optimally organize its disinfection program and reduce the presence of excess toxic Disinfection By Products (DBPs).

Q: How might chlorinated water affect luminescence?

A: Chlorine is usually introduced into drinking water systems in order to avoid bacterial contamination. Since luminous bacteria used in the assay are also sensitive to this treatment, Sodium Thiosulfate is included in the assay buffer to dechlorinate the sample before adding the bacteria.

Q: Why is there a control in each assay?

A: Readings of the negative control are needed in order to obtain the background reading of the cells without the sample. In addition, a set of positive controls is run in order to calibrate the system and provide the proper “translation” of light units to carbon equivalent units.

Q: Can I "play around" with the volumes of bacteria, buffers and other assay conditions?
A: No. It is extremely important to follow the test protocol instructions to the word. Since the test is very sensitive, any seemingly minor variations result in poor reliability.

Q: Can I reuse the provided test vials?
A: Due to the high sensitivity of the assay, care should be taken to keep all vials, plastic tips, and pipettes extremely clean. Do not reuse test vials and do not wash glassware pipettors or pipette tips with detergent, acid, or solvents.

Q: What is the shelf life of the reagents?
A: The shelf life of the freeze dried bacteria is one year when stored in a deep-freezer (-10°C to -20°C). Reagent should not be stored in a self-defrosting freezer, which defrosts by warming up periodically. The assay buffers should be stored in a regular refrigerator (~4°C) and under no circumstances should they be frozen.

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