NOVEL SENSITIVE SHORT-TERM
TESTS FOR WATER QUALITY

CheckLight Biocide Activity On-Site Test

CheckLight Biocide Test kit

Reagents provided:

I. Stoppered vials containing freeze-dried culture of *P.leiognathi* SB.
II. Concentrated Assay Buffer.
III. Hydration buffer.
IV. Storage buffer.
V. Empty test vials.

Optional additions (provided seperately):

- Distilled water (100mL, 500mL, 1 Liter).
- Insulating styrofoam case for carrying out field tests.

Equipment required:

- 10-1000µL pipettor and tips.
- Luminometer.

CheckLight Biocide Test

Introduction

Microbial problems associated with industrial cooling water and boiler systems, fuel storage tanks, pulp and paper slurries, metal-working fluids etc, are caused by algae, fungi, and bacteria. Many different bacteria species may exist in these systems and cause plugging, fouling, corrosion and may be a source of infectious disease.

To prevent these problems different kinds of biocides are added to the water on a routine basis. In order to ensure their continuous and efficient action it is highly important to periodically monitor their activity.

The commonly applied test for biocide activity determines the effect of the biocide on the viability of a few selected bacterial strains. This test is tedious and requires 24-48 hours to obtain results.

The CheckLight biocide test overcomes these problems and offers a short and simple way to measure biocide activity. The test is based on a lyophilized culture of marine luminous bacteria. The hydrated bacteria are mixed with the water in question and the level of luminescence developed after 5-10 minutes reflects the effect of the biocides on the viability of the luminous bacteria. Since common strains of *E.coli* and *Pseudomonas sp.* were found to exhibit a similar sensitivity pattern, this luminous bacteria-based test offers a rapid, reliable and economical method to determine biocide activity.

Rationale of test

The viability and vitality of luminous bacteria are manifested by high luminescence emission. Biocides that affect cell respiration, cell's integrity, and especially membrane function, have a strong effect on the ability of the luminous bacteria to emit luminescence. Using a luminometer, one can accurately measure the decrease in luminescence. Hence, by simply comparing the luminescence level obtained in a biocide-containing sample with that obtained in the control (clean water) vial, one may determine the activity of potent concentrations of a broad range of biocides.

All the commercial biocide preparations tested with CheckLight's Biocide Activity Test were found to be active down to concentrations of 1-10 ppm. In the case of biocides whose active range is 30-100 ppm, one may first dilute the biocidic agent to establish the expected degree of luminescence inhibition at the required concentration, and then proceed to test the biocide-containing sample.

CheckLight Biocide Test Reagents

Biosensor: A freeze-dried preparation of the luminescent marine bacterium *P. leiognathi* SB. The shelf life of this reagent is one year when stored in a deep-freezer (-14° – -20° C). Reagent **should not** be stored in a self-defrosting freezer, which defrosts by warming up periodically.

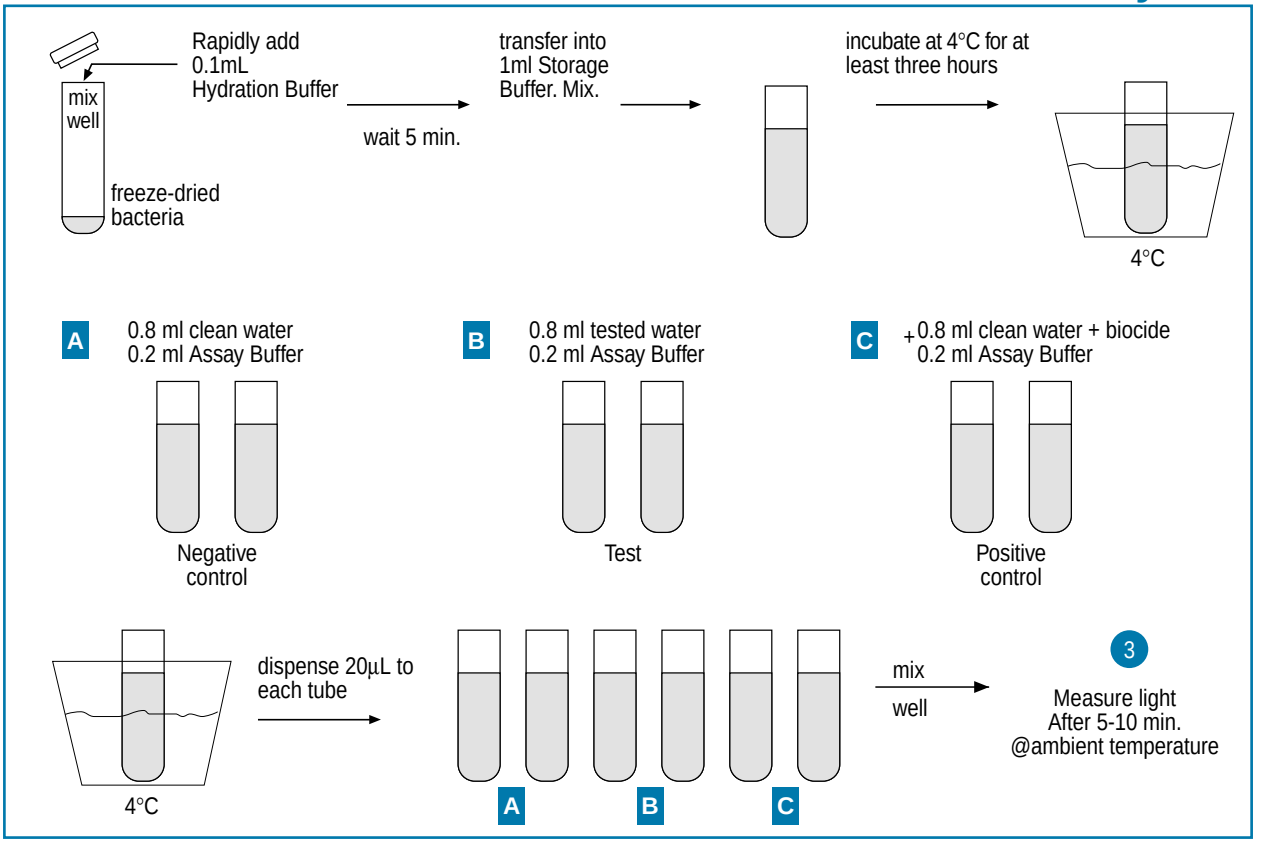
Preparation: once reconstituted with Hydration Buffer, reagent should be incubated 5 minutes at ambient temperature and then quickly and carefully transferred into Storage Buffer and mixed well. Reagent suspension may be used immidiately, however, for optimal and reliable results it is essential to incubate for **at least three hours** at 4°C before use. To minimize temperature changes when removing tube for testing it is recommended to store the tube in a water-filled beaker. Avoid unnecessary exposure to ambient temperature. Under these conditions aliquots of the reagent may be drawn repeatedly for up to 7 days.

Important information about the CheckLight Toxicity Test:

Due to the high sensitivity of the assay, care should be taken to keep all vials, plastic tips, and pipettes extremely clean. **It is recommended to wash the tips several times with clean water before use.** Do not reuse test vials and do not wash glassware pipettors or pipette tips with detergent, acid, or solvents.

Work under aseptic conditions to avoid contamination of reagents.

Biocide Activity Test



Before you begin - please follow carefully:

The luminous bacteria chosen for the test are more sensitive than microorganisms commonly found in water. Hence, the tested water needs to be diluted before being assayed.

For each new biocide agent used, it is vital that you first determine its luminescence inhibition profile:

1. Prepare a set of double dilutions of the biocide agent in (1:5) diluted Assay Buffer in clean water. Plan the dilutions to start from the applied biocide concentration down to 200 fold lower (e.g., from 50 ppm down to 0.25 ppm). Leave two vials without added biocide (i.e., control).
2. Carefully dispense 20µL of biosensor suspension to each of the vials in the dilution set.
3. Record luminescence after 5 minutes.
4. Determine the dilution fold, or the concentration of biocide, which led to **~90%** inhibition of luminescence compared to the control.

This is the concentration of biocide to be added to **vial C** (see scheme).
This is also the dilution fold of the tested sample (**vial B**) (see scheme).

You may now proceed and test the activity of this biocide agent in your samples.

Example:
If the control (no added biocide) gave 20000 Light Units, and the vial holding 1ppm of biocide gave 2000 Light Units (in a dilution set starting from 100ppm), then you need to add 1ppm biocide to vial C, and 0.01 ml of the water sample tested to vial B (=100 fold dilution).

Note: you only have to perform this process once for each new biocide agent. If you are using a combination of more than one biocide, prepare a dilution set from your applied cocktail.

Test Procedure

For each water sample tested you will need:

120µL biosensor solution.

1.2mL concentrated Assay Buffer.

6 empty vials (test and controls).

Standard clean water.

Before assaying questioned water, prepare the following:

• **It is strongly recommended to perform tests in duplicates.**

A - Add to first empty vial: 0.8mL of **clean** water (control) and 0.2mL Assay Buffer. Mix by pipetting up and down.

B - Add to second empty vial: 0.01-0.8mL of **tested** water (depending on required dilution), 0.2mL Assay Buffer, and **clean** water up to 1mL. Mix by pipetting up and down.

C - Add to third empty vial: 0.2mL Assay Buffer, biocidic agent at pre-defined concentration and **clean** water up to 1mL.

You may now proceed with the test:

1. Rapidly transfer 20µL of suspended cells into each of the 6 test vials. Mix.
2. Incubate vials at ambient temperature (18°-28°C).
3. Measure obtained luminescence in vials after 5-10 minutes.
4. Potent concentration of biocide should result in at least 50% reduction in luminescence level (B) as compared to the negative control (A). Result obtained in (C) reflects the maximal activity (i.e., luminescence inhibition) of the biocidic agent at the concentration tested.

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