NOVEL SENSITIVE SHORT-TERM
TESTS FOR WATER QUALITY

CheckLight BOD Test

CheckLight BOD Test kit

Reagents provided:

I. Stoppered vials of freeze-dried luminous bacteria.
II. Concentrated Assay Buffer.
III. Concentrated stock solution of carbon cocktail - standard (5 mg/mL).
IV. Hydration Buffer.
V. Empty test vials.
VI. Empty bottle for buffer dilutions.

Optional additions - provided seperately:

- Ultra pure water (100mL, 500mL, or 1Liter).
- Cork-screw 10mL glass tubes (for boiling tested water samples).
- 10N HCl (10x concentrated). 10N NaOH (10x concentrated). 2 M KH_2PO_4 buffer, pH 6.5 (2x concentrated)

Equipment required:

- 10-1000µL pipettors and tips
- 5mL, 10mL glass pipettes
- Repeat pipettor
- Luminometer (minimal sensitivity of 1 fmol ATP).

CheckLight BOD Test is recommended for:

Sewage
Reclaimed water
Industrial water effluents

CheckLight BOD Test

The principle of the test

The Biochemical Oxygen Demand (BOD) test aims to determine the concentration of oxidizable and biodegradable organic compounds in water.

CheckLight developed a unique technology for determination of BOD in water in a 2-3 hours long procedure. The active reagent is a freeze-dried preparation of *Vibrio harveyi*. The non-assimilable organic compounds in water (including carbohydrates, proteins, and complex nutrients) are first hydrolyzed by a mild acid pre-treatment (1N HCl, 60 minutes at 100°C). This treatment breaks down polymers into assimilable oligomers and monomers. Once exposed to the treated water sample, the hydrated bacteria will undergo prompt induction of luminescence providing 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 organic compounds can be determined within 2-3 hours. Comparative studies have found a high correlation between the standard 5 days long procedure and the CheckLight BOD test (Figure 1).

In addition to the clear advantages of rapidity and simplicity, the test has additional benefits:

1. Acid hydrolysis of the tested water converts all the water flora into biodegradable nutrients (a potential oxygen consuming nutrient source not measured in the standard BOD method).
2. The test determines only the consumption of 0_2 due to bio-oxidation of organic carbon sources (rather than reduced inorganic compounds utilized by some litho-autotrophic bacteria).
3. The high sensitivity of the test allows extensive dilution of the sampled water before testing, thus eliminating possible inhibitory effects stemming from sample turbidity or the presence of toxic agents.

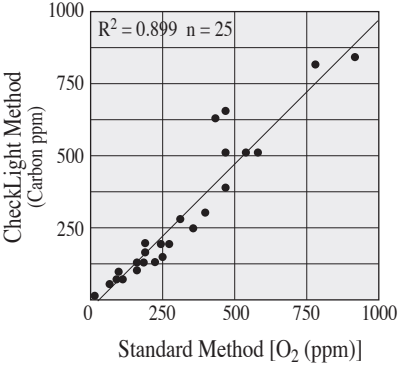


Figure 1: Correlation between the standard 5 days long BOD test and the CheckLight test.

Calculating the BOD value of the tested water sample:

1. Follow the procedure on the back page.
2. Calculate the average reading of the negative controls (ANC) and Standard Deviation (SD).
3. Subtract the ANC from all readings.
4. Calculate the minimal concentration of the tested water (in %) that exhibited an increase of (3xSD) in luminescence over the control. Note that the water concentration in vial #1 – 10%; vial #2 – 5%, etc.
5. Determine the (3xSD) of the Carbon Cocktail Standard by graphically plotting the luminescence obtained in each sample against its concentration.
6. The (3xSD) values obtained for the tested sample and the standard solution are defined as having an equivalent BOD value.

   Thus, for example, if 10% water and 20ppb Carbon Cocktail Solution were defined as the (3xSD) concentrations, the BOD of the water in question is said to contain 20ppb x 10 = 0.2ppm Carbon equivalent units.
7. In order to convert carbon equivalent units into the commonly used BOD units (μg glucose/Liter), one should run several samples in both standard and CheckLight methods to determine the correlative coefficient factor for all future tests with that water source. Alternatively, one could regard the carbon equivalent units as arbitrary units for comparison between BOD values obtained from different water sources.
8. **Suspected signs of toxicity**: when luminescence levels obtained in the first vials of the dilution set are lower than those recorded in the more diluted samples, and/or the spiking set (step 9 in test procedure) reveals a non-linear response. Various curves may appear when plotting sample dilution versus luminescence, depending on the level of BOD and concentration of toxicant.

Sample collection and storage

Sampling follows the same proceduce as for the standard method.

CheckLight BOD Test Reagent

A freeze dried preparation of an isolated variant of the marine luminescent bacteria *Vibrio harveyi*.

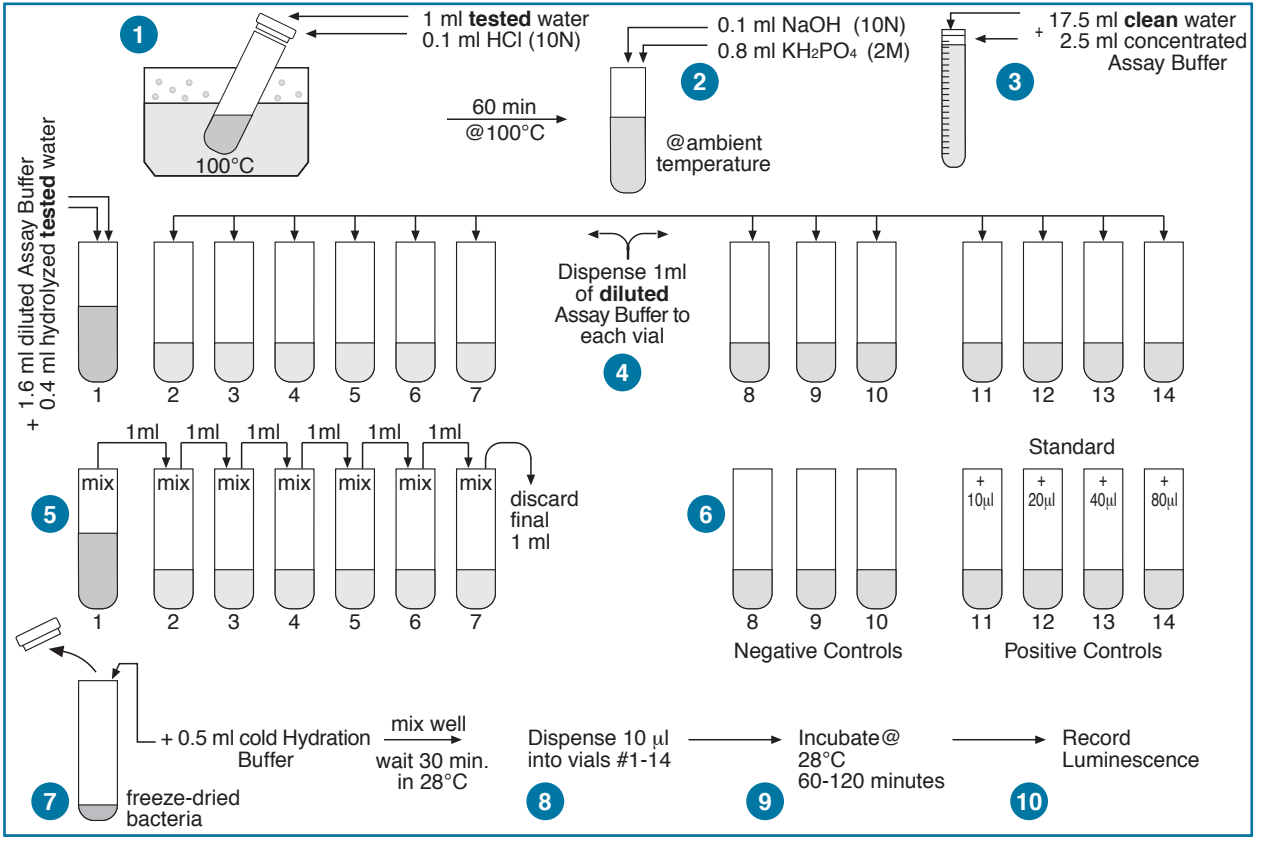
The shelf life of this reagent is one year when stored in a deep freezer (-14° C). Reagent should not be stored in a self-defrosting freezer, which defrosts by warming up periodically. Once hydrated in Hydration Buffer, the suspended cells can be used within 4 hours, if kept on ice or at 4°C.

Important information about the CheckLight BOD 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. Do not wash glassware pipettors or pipette tips with detergent, acid, or solvents.

Work under aseptic conditions to avoid contamination of reagents.

BOD Test


1
1 ml **tested** water
0.1 ml HCl (10N)
100°C
60 min
@100°C
2
0.1 ml NaOH (10N)
0.8 ml KH_2PO_4 (2M)
@ambient temperature
3
+ 17.5 ml **clean** water
2.5 ml concentrated Assay Buffer
+ 1.6 ml diluted Assay Buffer
0.4 ml hydrolyzed **tested** water
1 2 3 4 5 6 7
Dispense 1ml of **diluted** Assay Buffer to each vial
4
8 9 10
11 12 13 14
5
1ml 1ml 1ml 1ml 1ml 1ml
mix
discard final 1 ml
6
Standard
+ 10µl
+ 20µl
+ 40µl
+ 80µl
Negative Controls
Positive Controls
7
+ 0.5 ml cold Hydration Buffer
freeze-dried bacteria
mix well
wait 30 min. in 28°C
8
Dispense 10 µl into vials #1-14
9
Incubate@ 28°C 60-120 minutes
10
Record Luminescence

Test Procedure:

Note: careful and accurate pipetting is essential!

1. Incubate 1 mL of the **tested** water in a screwed-cap borosilicate tube containing 0.1 mL HCl (10N). Loosely screw back the cap and incubate the tube in a beaker containing boiling water for 60 minutes (keep the water in a boiling state throughout the incubation period).
2. Cool down the tube and add sequentially: 0.1 mL of 10N NaOH, 0.8 mL of 2M KH_2PO_4 (pH 6.5). Mix well. Make sure pH is ~7.0.
3. Dilute the concentrated Assay Buffer (1:8) in **clean** nutrient-free water (use the provided empty bottle for storing the diluted buffer. The final volume of diluted Assay Buffer depends on the number of samples and dilutions tested).
4. Set 14* clean vials in a rack. Add 1.6 mL of the diluted Assay Buffer to the first vial and 1mL to the rest of the vials.
5. Add 0.4 mL of the treated water sample to the first vial (note - up to this stage the tested water was diluted 10 folds).
6. Mix the first vial by pipetting up and down and transfer 1 mL from the first vial to the second vial (the first double dilution). Repeat this step 5 times (note- the tested water has been diluted ~ 600 times). Discard 1 mL from last dilution vial.
7. Leave 3 vials as negative control samples (holding diluted buffer only).
8. The last four vials will serve as positive controls: prepare 1 mL of Carbon Cocktail Solution diluted in Assay Buffer to a final conc. of **5ppm** (freshly prepared for each experiment). Dispense 10, 20, 40, and 80μL to 1st, 2nd, 3rd, 4th vials to yield 50, 100, 200, and 400ppb in each, respectively.
9. Hydrate the freeze-dried bacteria (kept in deep-freezer until use) with 0.5 mL cold Hydration Buffer; mix well with Vortex (remove stopper before mixing). Leave for 30 minutes at 28°C.
10. Using a repeat pipettor dispense 10μL into each vial. Mix well.
11. Measure obtained luminescence after 60-120 minutes (or when the two lowest concentrations of the standard solution emit significantly higher luminescence (>2xSD) than the negative control).
12. Calculate the BOD value of the tested water sample as explained on page 5.

* The number of vials (i.e., dilutions) chosen may be more or less than 7, depending on the suspected concentration of BOD in the sample.

Bioluminescent Diagnostic Reagents
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