ALGE-BART™
Standard Operating Procedures

DBGSOPO5

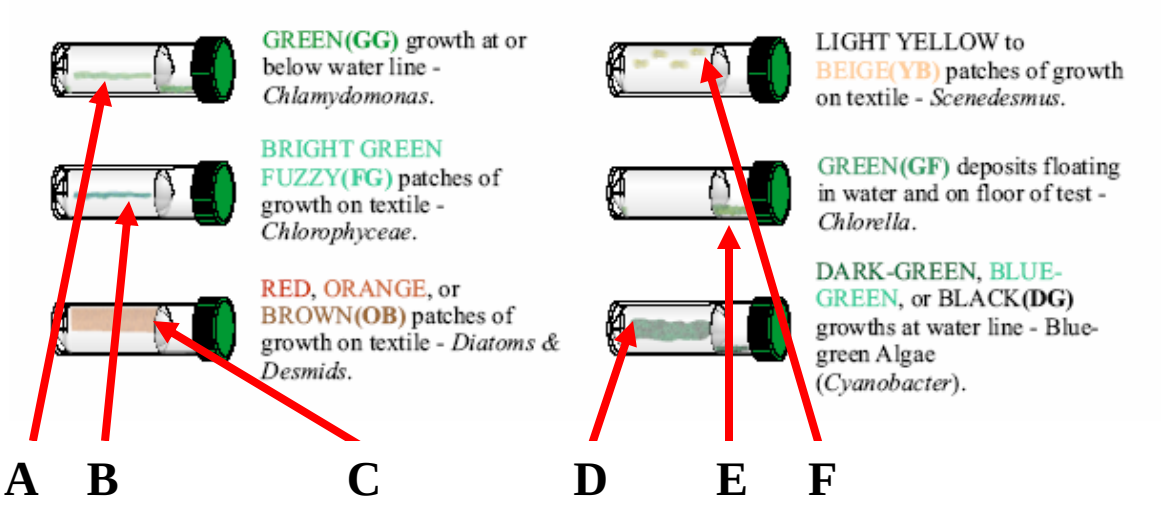
This test for the presence of algae in water and waste waters is based upon the ability the algae present in the sample to grow under illuminated conditions in a nutrient enriched environment. To achieve this, the ALGE-BART has to provide a range of conditions that would be suitable for the various species of algae that might be present and active in the sample. To affect this then it is particularly important to provide the correct level of illumination to the ALGE-BART tester. Some algae respond best to very low levels of light while others to relatively high intensity levels. Also important is the type of light being used since algae tend to respond better to a natural daylight. Unlike the other BART testers that can all be undertaken in the dark, this test is sensitive to the type of light being used. It is important to follow the specifications laid out in this standard operating procedure (SOP) for the ALGE-BART bearing in mind the operator of the test may find it beneficial to modify the illumination levels to optimize the detection of the algae in the samples being used.

Theoretical

Algae are simple plants having a simple cell form not differentiated into tissues. As a result algae are normally small growing either attached to surfaces (sessile) or floating freely in the water (planktonic). Water samples are more likely to contain the planktonic type of algae unless the attached algae have been released into the water through some from of disturbance that could have been caused by changes in the patterns of water flow or physical agitation. It is therefore important to consider whether the objective is to examine for planktonic algae only (in which case the water sample should be satisfactory) or to examine for both planktonic and sessile algae in which case care would need to be taken to assure that the sampled site was agitated physically or hydraulically before the sample was taken. Since the algae have a tendency to stratify within the sample it would also be important to agitate the sample before setting up the ALGE-BART.

The concepts used in the ALGE-BART tester are based upon a crystallized dried nutrient pellet set into the base of the inner tester. Here, the nutrient gradually diffuse into the water that is dominated phosphorus with a more minimal supply of nitrogen. This was done to encourage the activity of the algae that are able to fix nitrogen. These nitrogen fixing algae often dominate in surface waters as algal blooms form and the natural nitrogen supply diminishes with the demands from the algal biomass. By creating nutrient diffusion gradients along the sample the objective is to provide a series of niches where different algae are able to grow and flourish. To further encourage the activity and growth of sessile algae, the lower two thirds of the inner tester in lined with a porous medium that presents a large surface area over which these algae can grow. Additionally the additional porous medium also provides a "shadow" in the water sample that lowers the intensity of the light and encourages the growth of those algae not able tolerate high levels of illumination. The basic patterns of growth which can appear as a result of algal activity vary depending upon the types of algae that are present and active in the sample. Figure one illustrates the six focal sites where growth where algal growth may be recognized over the incubation period that can last as long as 25 days depending upon the size of the algal population.

Figure One, Focal Sites of Algal Growth in the ALGE-BART tester.



There are six different focal growth sites for the algae active in the ALGE-BART. They are: (A) as a grass green lateral line of growth slightly below the water level on the porous medium with some green growth on the floor of the tester; (B) as a bright green but fuzzy line laterally along the porous medium with some patches below: (C) patches of discoloration commonly in the range from orange to brown through red occur in the porous medium but above the water; (D) light yellow or beige patches are observed in the porous medium above the water line but they tend to be feint and difficult to recognize; (E) here the porous medium remains white but the water sample itself become green with both flocculant growth and deposits on the floor of the tester; and (6) a lateral growth occurs along water level interface with the porous medium that rapidly goes to a dark green, a blue green or goes black.

Each of these six reactions relate to particular groups of algae and generate two sets of data: (1) the time lag (commonly in days) to the first observation of algal growth gives an indication of the population; and (2) the reaction patterns observed indicates the types of algae that are dominating in the water sample. These findings are summarized in the interpretation section of the DBGSOPO5 document.

Standard Operating Procedure

To undertake the examination for algae in a water sample using the ALGE-BART tester the following materials are required:

1. A field ALGE-BART sealed in an aluminum foil pouch.
2. Water sample that has been collected in a sterile container.
3. A pipette or measuring cylinder that is graduated and would allow 15mL of sample to be dispensed
4. Clean dry surface on which the test can be monitored.
5. Source of light to ensure that any algae present in sample and active in the tester would be able to grow.

It should be noted that the ideal surface upon which the ALGE-BART testers will be placed for monitoring should be either white or of a light color that would allow any light to be reflected back. Black and dark surfaces should be avoided for this test. It is also recommended that the operator preparing the ALGE-BART should wear latex gloves to ensure no direct skin contact with the sample.

The source of light for the growing of the algae in the sample may come from any one of these possible sources. These may be:

A. Northern natural light supplemented with either option B or C to assure 24hour a day illumination. If natural light is used without supplementation then there is a likelihood that the growth of the algae will become delayed and the time lags (days) to the observation of growth would be lengthened.
B. Tungsten light can be used preferably using a 40watt lamp placed at no closer than 60cms and no greater than 100cms away from the tester. Note that significant heat can be generated from tungsten lights and this could cause localized heating unless the area in which the test is being conducted is well ventilated.
C. Fluorescent light can be used but only if it is a natural or daylight type of light. For this testing the lights may be placed within the range from 150 to 200 cms above the testers with a wattage not exceeding 80watts, or at least 40cms to the side and slightly above the tester.

Note that algae vary considerably in the light intensity and wavelengths. This would mean that the conditions may be suitable for the growth of some of the algae in the sample but not necessarily all of them. For example sessile algae growing close to, or in, sediments and soils tends to have a lower tolerance for high light intensities and too much illumination may actually suppress their growth in the tester. To compensate for that then

the distance to the lights should be placed at the greatest distance listed above for sessile algae while for plankton then the shortest distance can be used.

The sequence for starting the test using a water sample and a field ALGE-BART tester is described below:

(i) Tear down the aluminum foil pouch protecting the ALGE-BART and place the tester down onto a clean dry surface.
(ii) Unscrew the outer green cap from the tester and lift this cap up. You will notice that the inner tester also comes up because it is clipped into the outer cap. Remove the inner tester from the outer cap and place the cap on the clean dry surface without turning it over and place the inner tester upright on a clean dry surface.
(iii) Label the outer cap with the information relevant to the test sample using a fine tipped permanent black marker.
(iv) Unscrew the cap from the inner tester and place this next to the outer cap without turning it over. Pipette or pour 15mL of the sample to be tested into the inner tester and immediately return the inner cap to the inner tester and screw down firmly.
(v) Clip the inner test inside the correctly marked outer cap for the sample and return the inner tester back into the outer tester and screw the cap down firmly.
(vi) The test is now ready to start. To initiate the test place the ALGE-BART charged with the sample on its side so that the water sample now flows to the full length of the tester. Note and record the time at which the test started.

It should be noted that the ball in the ALGE-BART fulfills the function of ensuring that the porous membranes covering the bottom two thirds of the tester do not collapse and does provide a suitable environment on which some of the algae will grow.

In order to minimize the rolling of the testers since they are placed on their sides, it is recommended that the testers be placed close together in a row. By leaning the tester on the large cap, the tester is angled down to the base by about 5^{o} which means the water being tested tends to “pool” towards the base of the inner tester.

Interpretation – Time lag (days) to Population

The shorter the time lag then the premise is that there are more active algal cells present in the sample and these will cause the time to the first detection of activity or growth to be faster. Thus the shorter the time lag then the greater the population. In Table one the time lag (left hand column) is given in days and the predicted population is shown in the right hand column as predicted active cells/mL in the sample. Commonly very active (aggressive) algal populations will have populations greater than are >600 pac/mL with populations under very eutrophic conditions exceeding 100,000 pac/mL (with the time

lag being of less than six days). Operators can us QuickPop found on the web site www.dbi.ca if greater accuracy is needed.

Table One, Relationship of time lag (days) to the active algal population (predicted active algal cells per mL or p.a.a.c/mL) in the water sample

Time Lag days	Predicted Population
1	2,511,886
2	1,258,925
3	630,957
4	251,189
5	125,893
6	50,119
7	25,119
8	12,589
9	6,310
10	3,981
11	1,585
12	794
13	501
14	316
15	251
16	200
17	158
18	126
19	100
20	79
21	63
22	50
23	40
24	32
25	25
26	20
27	16
28	13
29	10

Note: the time lag only goes to 29 days and a P.a.a.c./mL or 10, for longer time lags the relationship between time lag and population lacks confidence since there is a probability that a significant fraction of the algal cells may be in a suspended state of animation and not able to adapt to the given conditions in the ALGE-BART using the DBGSOPO5 protocol.

Interpretation – Reaction patterns to algal groups present in sample.

While the time lag gives a prediction of the population in the water sample being tested, the reactions observed that triggered the time lag along with secondary reactions indicate which types of algae are dominant in the sample and are able to be active in the ALGE-BART tester. Figure one shows the reaction patterns that are recognized as positive for the detection of algal activity. Each of these reactions represent a different and distinct group of algae and secondary reactions overlay to indicate that relative dominance of these different types of algae. Table two lists the six common reaction types and primary interpretation.

Table Two, Interpretation of reaction types for the ALGE-BART

Type	Color type	Description	Major algal genera
GG	Grass green	Generally as a continuous lateral growth occurring at the water line	*Chlamydomonas*
FG	Fuzzy green	Patches of green growth at the water line but with fuzzy edges and sometimes indistinct	Many genera in the *Chlorophyceae*
OB	Orange to brown	Growth tends to be above the water line in the porous media as orange, red or brown patches.	Commonly associated with diatoms and desmids
YB	Yellow to Beige	Fuzzy patches of growth often above the water line may appear to be green-yellow initially but rapidly become yellow or beige	*Scenedesmus*
GF	Green flocculant	Much of the growth here occurs within the water as green flocs and/or deposits while the porous medium remains white.	*Chlorella*
DG	Dark green	Growth begins a lateral green at the water level but the color rapidly goes darker and can even turn black. Dark green dense floc can also occur on the floor of the tester.	Blue-green algae, *Cynaobacteriaceae*