GEORGIA EPD LONG-TERM BOD TEST SPECIFICATIONS Revised January 2005

There are two types of requirements for the successful performance of a long-term Biochemical Oxygen Demand (BOD) test - 'laboratory procedures' and 'test specifications'. Laboratory procedures are those that should be followed consistently from test to test. By comparison, test specifications often vary from test to test depending on the information needed by modelers to develop TMDLs and NPDES permit limits.

LABORATORY PROCEDURES

Laboratory procedures cover familiar activities like: sample handling, proper instrument calibration, maintenance of incubator temperatures, performance of dilutions and seedings, appropriate reaeration techniques, standard analytical methods, glassware and chemical preparation, and so on. These activities should be performed consistently from project to project, and unvaryingly from sample to sample. The Georgia EPD long-term BOD laboratory procedure should be followed for all long-term BOD tests performed for, or at the request of Georgia EPD. These can be found in the following manual:

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EPD. The Amplified Long-Term BOD Test: Protocol/Procedure and Test Specifications,
Environmental Protection Division, Atlanta, Georgia, November 1989, 31 pp.
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Two additional long-term BOD procedure documents may be consulted for reference and comparison. They include Standard Methods 20th Edition, and the Long-Term BOD procedure used by the North Carolina Department of Environment & Natural Resources.

APHA, AWWA, WEF. Standard Methods for the Examination of Water and Wastewater, 20th Edition.

NCDENR. SOP Section 5. Long-Term BOD Procedure. North Carolina Department of Environment & Natural Resources, Division of Water Quality, December 1995. (North Carolina standard lab test procedure.)

All three documents have been incorporated as *.pdf files into the Help menu of the LtBod V3.0 software package.

TEST SPECIFICATIONS

Test specifications differ from laboratory procedures in that specifications often change from project to project, and even from sample to sample, depending on the information needs for TMDL and NPDES permit development. These specifications cover aspects like: test duration in days, number and spacing of dissolved oxygen (DO) measurements, number and spacing of nitrogen sub samples, requirements for dilution and seeding, sample treatments needed like filtering and nitrogen inhibition. Typical test specifications required by the Georgia Environmental Protection Division (EPD, or the Division) are presented below. Prior approval from the Division would be required for any deviations from these specifications.

Test Integrity

Continuity of a given test should never be interrupted. If this occurs it may be necessary to restart the test with a new sample.

Analytical instruments should be calibrated according to manufacturer's specification. The calibration record for each instrument should be included in the laboratory record.

Incubator temperature should be controlled at 20 °C plus or minus 0.5 °C.

Immediate sample setup. Do not store samples or delay setup activities once samples have been delivered to the laboratory.

Sample setup time is the official reference time for all calculations and analyses performed on lab results. It represents the time of the initial dissolved oxygen measurement and chemical sub sample. The setup time must be determined and clearly recorded in the lab record for each test. If the setup time is not recorded it will be necessary to restart the test with a new sample.

Initial dissolved oxygen reading and nitrogen sub sample (required at setup time) should never be forgotten or omitted. If this occurs it will be necessary to restart the test with a new sample.

Careful handling of the monitored sample is necessary at all times to minimize, and hopefully prevent, inadvertent gain or loss of dissolved oxygen from the sample by agitation or unnecessary exposure to the atmosphere, especially when outside the incubator for measurements.

20 minutes is the maximum time a sample can be outside the incubator for measurement or chemical sub sampling.

Test Duration

The formal duration of each long-term Bod test should be 120 days. This means 120 days "more or less". In the event day 120 falls on a weekend or holiday the test may be terminated on the nearest working day. A test duration less than 120 days requires prior approval by the Division.

Required Labware

Monitored bottle used for DO measurements should be 2 liters or greater in volume and fitted with a ground glass stopper. A water seal should be maintained at all times except when out of the incubator and opened for measurement. Bubbles are not allowed in the monitored bottle when sealed and stored in the incubator.

Reservoir bottle for makeup water lost from the monitored bottle should be 1 liter or greater in volume. A ground glass stopper is not required, however the reservoir bottle should be covered at all times to prevent contamination. The monitored and reservoir bottles for a given test should be kept in similar conditions, preferably side-by-side.

Frequency of Dissolved Oxygen Measurements

In general, there should be a minimum of thirty (30) dissolved oxygen measurements for a test of 90 days duration, or longer. Measurements should be taken more frequently near the beginning of the test when DO is declining fast in the monitored bottle, and less frequently when DO decline slows down.

With the exception of strong fast-acting waste waters, the frequency of DO measurements should follow the following schedule:

Week 1 (days 1-7):	once a day
Weeks 2-3 (days 8-21):	once every other day
Weeks 4-6 (days 22-42):	once every third day
Weeks 7-17 (thru the end):	once a week

Measurements may be shifted forward or backward in time to avoid weekends or holidays **with the exception of those in Week 1**. The first few days are critical for test acclimation and subsequent data analysis. Therefore, daily measurements should be made during the first week. In addition, some strong waste water samples may exhibit rapid oxygen depletion during the first two weeks; this may necessitate more frequent DO measurements during the initial period. If excessive depletion occurs, then measurements should be made twice a day or more, and reaerations should be performed frequently enough to keep DO above 3 mg/l in the monitored bottle. (These provisions will be considered further in the section below on sample dilutions.)

- Note: The initial dissolved oxygen measurement at setup time, the start of the test, is vital and should not be forgotten or omitted. Without a setup or initial dissolved oxygen the test will have to be restarted with a new sample.
- Note: Day 5 dissolved oxygen measurements are important and should not be skipped.

Frequency of Nitrogen Series Sub Samples

In general, there should be a minimum of ten (10) nitrogen series sub samples for a test of 90 days duration, or longer. Samples should be taken more frequently near the beginning of the test when ammonia conversion in the monitored bottle is rapid or delayed, and less frequently when ammonia conversion slows and approaches its maximum.

- TKN sub samples should be taken only at the beginning and end of the test. This helps to reduce excessive loss of sample volume during the test.
- Ammonia and nitrite-nitrate samples should be taken on days: 0, 5, 10, 15, 20, 30, 40, 60, 90, and at the end of the test.

Sample Temperature Measurements

The incubator temperature should be controlled at 20 ℃ plus or minus 0.5 ℃.

The sample temperature recorded during DO measurements should be the actual sample temperature in the monitored bottle. It should not be the thermostat temperature in the incubator or any other general lab temperature. Since the incubator temperature should be controlled at 20 °C plus or minus 0.5 °C, each sample temperature should fall within this range. If not, then something is amiss and should be noted in the 'Remarks' section of the data sheet or grid. If out-of-bounds incubator temperatures persist for more than 2 or 3 days, action should be taken to correct the problem. Persistent incubator temperature excursions may require that the test be restarted with a new sample. Restarting the test requires prior approval by the Division.

Sample Reaeration at Minimum DO

Dissolved oxygen in the monitored bottle should not be allowed to fall below 3 mg/l. When this condition occurs, or is anticipated, the combined sample - the contents of both the monitored and reservoir bottles - should be mixed and reaerated according to procedures contained in the Division's *Amplified Test* manual.

Note: Remember to measure DO immediately after reaeration, before the sample is returned to the incubator. Record the reaerated DO in the lab test data. If reaerated DO's are not recorded the test will have to be restarted with a new sample.

Sample Reaeration after Chemical Sub Sampling

If the volume of any chemical sub sample taken from the monitored bottle during the test exceeds one percent of the monitored bottle volume, the entire sample (monitor and reservoir bottles) should be combined and reaerated according to procedures contained in the Division's *Amplified Test* manual. If the sub sample is less than one percent of the monitored bottle volume, the lost amount should be replaced from the reservoir bottle without total sample reaeration.

Note: Remember to measure the DO concentration immediately after reaeration, before the sample is returned to the incubator, and record the reaerated DO in the lab test data. If the reaerated DO concentration is not recorded, the test will have to be restarted with a new sample.

Sample Dilution

It is unlikely that natural water samples will require dilution. However, it may occasionally seem necessary to dilute strong fast-acting waste waters. In the event that a sample must be diluted, the

dilution water selected must be approved by the Division before test initiation and conform to criteria presented in the Division's *Amplified Test* manual, for example:

- Source of dilution water
- Dilution water collection, storage, and monitoring requirements
- Dilution water preparation, treatments, seeding, and testing

Note: (1) The Division recommends that long-term Bod samples be undiluted. Any sample dilutions require prior approval from the Division.

- (2 For strong samples that would appear to need dilution, the Division instead recommends more frequent DO measurements during the first fifteen days of the test. This may mean measuring DO more than once each day initially.
- (3) If a sample must be diluted, then a separate and complete long-term Bod test will be required on the dilution water itself. The dilution water test should meet all procedures and specification that apply to any other sample.

Sample Treatments

Sample 'treatments' are those procedures applied to a sample to provide additional information. Any treatment performed must conform to criteria presented in the Division's *Amplified Test* manual and requires prior specification or approval by the Division. Common treatments include:

- Filtration is occasionally required to quantify the effect of suspended particulate matter or isolate the activity of algae or bacteria. Typically, a sample will not be filtered without prior specification by the Division. Filtered versus unfiltered samples comprise a 'paired test' requiring special considerations described below.
- *Nitrification Inhibition* is occasionally required to quantify the amount of nitrogeneous Bod in a sample. Typically, a sample will not be inhibited without prior specification by the Division. Inhibited versus uninhibited samples comprise a 'paired test' requiring special considerations described below.
- Seeding may be needed when a sample does not contain enough organisms or nutrients to support the proper exertion of Bod. This typically occurs in dilution water or in waste water collected after disinfection. Sample seeding will not typically create the need for a 'paired test'.
- Dechlorination will be required for waste water samples taken after disinfection by chlorine. It is preferable to collect waste water samples prior to chlorination. Sample collection after chlorination requires prior approval by the Division. Sample dechlorination will not typically create the need for a 'paired test'.

Special Test Conditions

'Paired tests' may occasionally be recommended. The necessity for paired tests occurs for split or duplicate samples, filtered versus unfiltered tests, nitrification inhibited and uninhibited tests, or diluted samples requiring a long-term Bod test on the dilution water itself. Each of these circumstances will require the data from one test to be compared, data point by data point, with data from the paired test.

Accordingly, paired tests should be conducted under identical laboratory conditions. Paired samples should be kept together as much as possible. All measurements, chemical sub samples, and reaerations on paired test samples should be performed as closely to the same time as possible.

Record Keeping

Clock times for all measurements should be reported in the 24-hour convention. Standard time (as opposed to daylight time) is preferred. However, daylight time will be acceptable as long as that fact is

reported in the laboratory record. During daylight time all recordings should be either daylight or standard; both daylight and standard times should not be intermixed.

Electronic data formats described and provided by the LtBod V3.0 software must be used for data recording and transfer to the Division.

Changes and Notifications

These are familiar or typical LtBod test specifications appropriate in most cases. Any departure from these, or others contained in the *Amplified Procedures* manual, would require prior approval from the Division. On a case-by-case basis, the Division may occasionally modify test procedures or specifications to address particular problems or information needs.

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