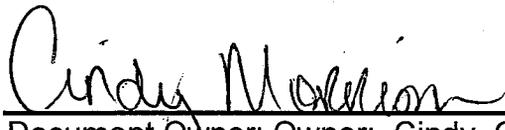


**STANDARD OPERATING PROCEDURE (SOP) FOR THE
DETERMINATION OF ULTIMATE BIOCHEMICAL OXYGEN DEMAND
(UBOD SURVEY)**

METHOD 5210C (PROPOSED), STANDARD METHODS, 20th EDITION

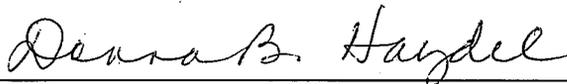
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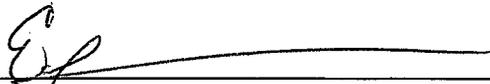
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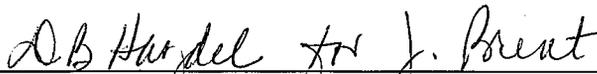
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Annual Document Reviews:

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2nd Review - 9/18/09 – Personnel changes, minor changes to blank acceptance criteria, minor changes to dilution procedure & calculation

3rd Review

4th Review

Changes Reviewed and Approved by:

	Analyst:	Supervisor:	Manager	QAO	Date:
1 st	_____	_____	_____	_____	_____
2 nd	_____	_____	_____	_____	_____
3 rd	_____	_____	_____	_____	_____
4 th	_____	_____	_____	_____	_____

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1.0 [Identification of Test Method](#)

This SOP describes the application of Standard Methods for the Examination of Water and Wastewater's Proposed Method 5210C (20th edition) in the LADEQ Laboratory Services Division, General Chemistry Unit. It is an extension of the 5 day Biochemical Oxygen Demand (BOD) test as described in 5210B (Standard Methods, 20th edition), with a number of specific test requirements and differences in application.

2.0 [Applicable Matrices](#)

The Ultimate BOD test is applicable to surface waters, waste water, and effluent.

3.0 [Detection and Quantitation Limits](#)

All results greater than zero are reported for use in modeling. There is no established PQL.

4.0 [Scope and Application](#)

- 4.1 The method has wide scope and applicability to all surface waters in general. The test measures the oxygen utilized for both the biochemical degradation of organic material and the oxidization of inorganic material such as sulfides and ferrous iron during a 60-day incubation period.
- 4.2 The results of this test plus results from other parameters such as TKN, TOC, and $\text{NO}_3\text{-NO}_2$ aid LDEQ engineers in modeling surface waters.

5.0 [Summary of Method](#)

- 5.1 The method consists of placing undiluted samples in airtight 2 L bottles and incubating at $20 \pm 1^\circ \text{C}$ for 60 days. Dissolved oxygen (DO) is measured (with probes) initially and intermittently during the test. From the DO versus time series, UBOD is calculated by an appropriate statistical technique.
- 5.2 A mixed standard of glucose and glutamic acid (GGA) is used to indicate that the test conditions are acceptable.

6.0 [Definition of Terms](#)

- 6.1 Analytical batch – An analytical batch is composed of 10 or less prepared environmental samples (extract, digestates or concentrates) and/or those samples not requiring preparation, which are analyzed together as a group using the same calibration curve or factor. An analytical batch can include samples originating from various environmental matrices.
- 6.2 GGA – Glucose-Glutamic acid, used as a laboratory control standard in this SOP and other BOD procedures.
- 6.3 Reservoir – the excess sample that is kept in its own bottle and is used to refill the 2L BOD bottle.

6.4 See QAM, Appendix B

7.0 Interferences and Pretreatments

- 7.1 Samples having pH >8.5 or <6.0 - Neutralize samples to a pH within the range of 6.5 to 7.5 with a solution of 10% sulfuric acid or 10N sodium hydroxide. The amount added must be measured, and the final pH recorded. (See section 14.2.8)
- 7.2 Samples containing residual chlorine compounds - In some samples chlorine will dissipate within 1 to 2 hr of standing in the light. For samples in which chlorine does not dissipate in a short time, destroy the residual chlorine by adding Na₂SO₃ solution (See dechlorination method, Appendix F).
- 7.3 Samples containing other toxic substances - Certain industrial wastes contain toxic metals. Such samples require special study and treatment.
- 7.4 Samples supersaturated with dissolved oxygen - Samples that contain more than 9 mg/L of DO at 20 °C may be encountered when warm water is cooled rapidly. Reduce the DO of supersaturated samples by placing the entire sample in a clean beaker and aerating for 3 minutes with house air. If the sample DO is still too high, repeat.
- 7.5 Samples with industrial or biological waste interferences – Industrial or biological waste in a sample can cause the DO to drop very rapidly. To avoid complete depletion of the sample, set up dilutions, as well as the undiluted sample. Prepare reservoirs for each dilution also.

8.0 Safety

- 8.1 While the toxicity or carcinogenicity associated with wastewater cannot be precisely defined, each sample should be treated as a potential health hazard and exposure should be as low as reasonably achievable. The toxicity or carcinogenicity of each reagent used in this method has also not been fully established and therefore should be treated as a potential health hazard. Exposure to these chemicals must be reduced to the lowest possible level by whatever means available. Cautions are included for known extremely hazardous materials.
- 8.2 Standard laboratory clothing (lab coat or apron), eye covering (safety glasses or goggles), and gloves shall be worn.
- 8.3 Material safety data sheets (MSDS) of all chemicals used in this SOP are available at a web site maintained by Cornell University. The link is: <http://hazard.com/msds/index.php>.
- 8.4 The following chemicals have the potential to be highly toxic or hazardous in concentrated amounts. For detailed explanations, consult the MSDS.
 - 8.4.1 Sodium hydroxide
 - 8.4.2 Sulfuric acid
 - 8.4.3 Sodium azide
 - 8.4.4 Potassium iodide

- 8.4.5 Potassium hydroxide
- 8.4.6 Sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$)

9.0 [Equipment and Supplies](#)

- 9.1 Balance -- analytical, capable of accurately weighing to nearest 0.0001 g.
- 9.2 1 to 2 L Glass Reservoirs with plastic screw caps - cleaned by vendor.
- 9.3 2 L BOD bottles – 2 L glass bottles with ground-glass stoppers. Before use in an analysis, bottles must be washed according to Appendix E.
- 9.4 Air incubator- thermostatically controlled at $20^\circ\text{C} \pm 1^\circ\text{C}$. All light is excluded to prevent the possibility of photosynthetic production of D.O.
- 9.5 Dissolved Oxygen Meter (YSI5100 & YSI159) and Probe - calibrated by Winkler Titration.
- 9.6 Dilution Water Container - 24 L Nalgene carboy rinsed with 10% HCl or H_2SO_4 . Swirl the jug and pour out the rinse. Rinse the carboy thoroughly with reagent water.
- 9.7 Accumet pH meter –capable of performing multipoint standardizations.
- 9.8 Pipets:
 - 9.8.1 Class A – 20 ml, 40 ml
 - 9.8.2 Graduated Disposable Transfer Pipet – 3 ml
 - 9.8.3 Glass Disposable Pipet – 1 ml - used for aerating reagent water.
- 9.9 Glass Graduated Cylinders – 1 ml, 10 ml, 100 ml, 1L
- 9.10 Glass Buret – 10 ml
- 9.11 Automatic self-zeroing Buret
- 9.12 Volumetric Flasks – 10 ml, 25 ml, 50 ml, 100 ml, 200 ml, 250 ml, 500 ml, 1000 ml, 2000 ml
- 9.13 Plastic Volumetric Flasks – 203 ml (for Winkler)
- 9.14 Plastic Disposable Amber Bottles – 300 ml (used for calibrating D.O. Meter)
- 9.15 Stir Plate
- 9.16 Beakers – 50 ml, 150 ml, 200 ml, 250 ml, 400 ml, 600 ml, 1000 ml, 4L
- 9.17 Ultra Zero Purified Air (“house air”)

10.0 [Reagents and Standards](#)

NOTE: Smaller/larger total volumes of any reagent or standard may be used based on expected analysis consumption. Be sure to use the correct proportions based on $m_1 \cdot v_1 = m_2 \cdot v_2$ calculation.

- 10.1 All reagents and standards must be prepared with reagent water. Each reagent container must be labeled with name of solution, unique solution ID (based on

reagent/standard logbook number and Std. ID #, i.e. B# 0xx-# xxx), date prepared, date expires, and analyst's initials. Additional information such as vendor lot number of concentrate, dilution factor (if used), amount of standard or reagent used, and true value can also be added. All chemicals used must be ACS-certified reagents or better. All standards shall be prepared using CRM. All reagents and standards information shall be entered into a logbook.

10.2 Preparation of Reagents

10.2.1 Hach* Nutrient Buffer Pillows 3L

10.2.2 "Nutrient" Dilution water – used for preparing "Nutrient" Dilution water blanks and GGAs

10.2.2.1 Fill a 24-L carboy with reagent water the day before the analysis. Put the carboy containing the water in the incubator overnight.

10.2.2.2 Aerate the reagent water with house air until the DO is between 7.5 ppm and 8.5 ppm. A 24 L carboy takes about 15 minutes of aeration to reach the desired DO. The DO must not exceed saturation for that temperature.

10.2.2.3 To aerate the reagent water, connect a 1 ml disposable pipette on the end of the tubing connected to the "house air" or an ultra pure zero-air cylinder. Place the pipette into the carboy and turn the air on.

10.2.2.4 While the reagent water is aerating, add one 3L nutrient buffer pillow. Adjust the airflow by turning the control valve to about 25 psi. Be sure the "nutrient" dilution water is well mixed. This "nutrient" dilution water expires daily.

10.2.3 "Aerated" water for dilution of samples and corresponding blanks

10.2.3.1 Prepare according to steps 10.2.2.1 thru 10.2.2.3. Do not add nutrients.

10.2.3.2 "Aerated" water expires daily.

10.2.4 Winkler titration reagents

10.2.4.1 Manganous sulfate solution –

10.2.4.1.1 AccuSPEC, 361 g Manganous Sulfate / 1000 ml. NIST traceable.

10.2.4.2 Alkali-iodide azide reagent –

10.2.4.2.1 In a one-liter volumetric flask, add 500 g NaOH (or 700 g KOH) and 150 g KI (or 135 g NaI) in reagent water and dilute to 1L with reagent water. Stir or invert to dissolve. Take extra precaution when adding the base because it will get very hot. Mix well. **Caution – strong base!** Heat will be generated when mixed with water. Cool before mixing with the NaN_3 solution.

- 10.2.4.2.2 Prepare the NaN_3 solution by dissolving 10 g NaN_3 in 40 ml of reagent water.
- 10.2.4.2.3 Combine the NaN_3 solution with the solution of NaOH and KI for a total of 1040 ml of reagent. Mix well, then store in amber bottle. This solution expires in 1 year.
- 10.2.4.3 Solution of 75 % v/v Sulfuric acid – Place 250 ml of reagent water in large beaker or glass cylinder. Slowly add 750 ml conc. H_2SO_4 (sulfuric acid). **Caution – strong acid!** Heat will be generated when mixed with water. Take extra precaution when adding the acid because it will get very hot. Mix well. Cool and then store. This solution expires in 1 year.
- 10.2.4.4 AccuSPEC, 2% W/V starch indicator
- 10.2.4.5 EMD, Sodium Thiosulfate Solution, Titrisol – Make 0.1 N $\text{Na}_2\text{S}_2\text{O}_3$ according to package directions, diluting to 1L. Prepare fresh monthly.
- 10.2.4.6 0.025N $\text{Na}_2\text{S}_2\text{O}_3$ Solution – Measure 250 ml of 0.1N $\text{Na}_2\text{S}_2\text{O}_3$ solution using a 250 ml volumetric flask and pour into a 1L volumetric flask. Rinse the 250 ml flask several times with reagent water and add the rinsings to the 1L volumetric flask. Dilute to the mark with reagent water. Prepare the 0.025 N solution weekly. Store in the dark.
- 10.2.5 Dechlorination Reagents:
 - 10.2.5.1 Dilution of 1 + 50 H_2SO_4 – Into a 100 ml volumetric flask with approximately 50 ml of reagent water, add 2 ml conc. H_2SO_4 . Dilute to the mark with reagent water. This solution expires in 1 year.
 - 10.2.5.2 Preparation of Potassium iodide (KI) solution – Into a 100 ml volumetric flask, dissolve 10 g KI in reagent water and dilute to the mark with reagent water. Store in a dark place. Discard if the solution turns yellow.
 - 10.2.5.3 Preparation of 0.0125 N Na_2SO_3 (sodium sulfite) solution – Into a 100 ml volumetric flask, dissolve 0.1575 g Na_2SO_3 in reagent water. (Prepare the day of dechlorination).
 - 10.2.5.4 Chlorine Test Tablets – commercially available.
- 10.3 Preparation of GGA (glucose-glutamic acid) Solutions –
 - 10.3.1 NSI BOD GGA standard solution - Into a 1 L volumetric flask, pipette 20 ml of concentrate. Dilute to the mark with reagent water. The resulting solution will be 150 mg/L each in glucose (dextrose) and L-glutamic acid. This results in a total dissolved reagent level of 300 mg/L. Smaller volumes may be prepared, i.e., 5 ml of concentrate in 250 ml, 2 ml of concentrate in 100 ml, or 4 ml of concentrate into 200 ml, etc. Prepare fresh daily.
 - 10.3.2 Preparation of 60 day working GGA solution as LCS - Prepare the glucose-glutamic acid (GGA) standard according to instructions. Add 40

ml of the prepared GGA standard solution of choice to a 2L volumetric flask of “nutrient” dilution water. Also add 20 ml to a 1L flask of “nutrient” dilution water. Dilute to volume with the aerated “nutrient” dilution water. Combine the two to make one GGA control in a clean beaker. Fill the 2-L UBOD bottle and use the excess as its reservoir. Two (2) GGA controls must be set up for the first batch in each UBOD survey set up. Subsequent batches must have one GGA control.

11.0 [Sample Collection, Preservation, Shipment, Storage, and Sample Rejection Policy](#)

- 11.1 This laboratory does not handle collection and preservation. See Section 11 of QAM.
- 11.2 Samples are shipped to the lab by common carrier or hand delivered.
- 11.3 For policy on chain of custody usage, see Section 11.5 of QAM.
- 11.4 Samples must be preserved according to 40 CFR Part 136 Table II.
- 11.5 Analysis must be made as soon as possible. When samples must be stored, they shall be refrigerated at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ until setup. Initial set-up and reading of the samples must be done within the 48-hr holding time from collection.
- 11.6 All samples received are analyzed, regardless of their condition. Any condition that violates the SOP for storage, shipment condition, etc. will be noted on the report; and the data must be flagged and qualified. Refer to QAM for data qualifiers.
- 11.7 Minimize the holding time to prevent sample degradation. Warm the chilled samples in the closed cubitainers to $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ before sample analysis. Rapid warming may cause D.O. to rise out of range.

12.0 [Quality Control](#)

- 12.1 A quality control program is required for this method. The requirements of this program consist of demonstration of laboratory capability, and the continuing analysis of laboratory blanks, field duplicates, and LCSs as an ongoing demonstration of performance. All Demonstrations of Capability (DOCs) must be documented using the appropriate Certification Statement Form. The analyst shall maintain performance records that define the quality of the data that are generated.
- 12.2 Demonstration of Capability
 - 12.2.1 The demonstration of capability requires 60-day analysis of one blank and four LCS replicates made with dilution water that has 12.5% nutrients added before aeration.
 - 12.2.2 Lab Control Standard (LCS) – An LCS must be analyzed at the beginning of this method to verify the meter’s calibration and acceptable instrument performance. An LCS must be analyzed with each analytical batch. The control limits for the GGA’s are 300 mg/L +/- 30.5.

12.3 Assessing Laboratory Performance

- 12.3.1 **“Nutrient”** Dilution water blank – The laboratory must analyze two “nutrient” dilution water blanks at the beginning analysis. Data produced are used to for calculation of GGAs. D.O. depletion of the “nutrient” dilution water blank must not exceed 0.5 ppm in 20 days, and 1.0 ppm in 60 days. Values that exceed the approved limits indicate laboratory or reagent contamination and actions must be taken to correct this before continuing the analysis.

13.0 [Calibration](#)

- 13.1 Calibration of the YSI meter is determined by using the Winkler titration method.
- 13.2 Two Winkler titrations are done and the results are averaged. The difference in the values of both bottles must be within the range of +/- 0.2 mg/L. The average is the set point for the meter’s calibration.
- 13.3 Check the calibration of the D.O. meter after every ten samples. If it drifts more than 0.2mg/L, recalibrate.
- 13.4 Calibration of YSI meter – see Appendix B.
- 13.5 Calibration of pH meter – see Appendix C.

14.0 [Procedure](#)

- 14.1 Complete these steps the day before setup:
- 14.1.1 Prepare **“nutrient”** dilution water as described in Section 10.2.2. Place the carboy of water in the incubator set at 20 ± 1 °C the day before setup. **NOTE:** If the reagent water system has just been sanitized, be certain system and lines are thoroughly flushed prior to collection of reagent water.
- 14.1.2 Prepare **“Aerated”** water as described in Section 10.2.3. This water is to be used only to dilute samples with initial low DO readings. If sample dilutions are made, blanks must also be made with the **“Aerated”** water.
- 14.1.3 Change the DO membrane if the current one has been in place for a week or longer.
- 14.1.4 Check that all reagents used for calibration are available and within expiration dates.
- 14.2 Initial Setup of a Survey - Complete these steps on the day of the analysis:
- Note:** The following steps must be done before analyzing any UBOD sample, but they do not necessarily need to be done in the order listed below. The only mandatory step is that the pH and DO meters must be calibrated correctly before any readings are made.
- 14.2.1 Batch the samples.
- 14.2.2 Remove the carboy of reagent water from the incubator. Aerate the entire 24L of reagent water. CAUTION: Aeration is not to be done until the day of analysis. See Section 10.2.2 for aeration instructions.

- 14.2.3 Perform a Winkler titration with the aerated dilution water. See Appendix B. Use the value obtained to calibrate the D.O. meter.
- 14.2.4 Calibrate pH meter according to Appendix C.
- 14.2.5 Prepare samples by bringing them to about 20°C. Do this by placing them either in a water bath or into the incubator.
- 14.2.6 While samples are warming, label all glassware and bottles to be used for the analysis. Label each 2 L BOD bottle and reservoir with the LIMS number, site number, and dilution factor, if it applies. Add a 2" stirring bar to each BOD bottle that will contain samples. Label a cup for each sample to be poured off to analyze pH and residual chlorine. The cup needs only a sample number. Label a 30 ml bottle for each sample, which includes the reading number, date, sample number, and the survey code for NO₃NO₂.
- 14.2.7 Record sample information in the BOD Survey sample logbook. For each sample, the sample number, site number, physical characteristics (color, consistency, etc.), initial pH and final, if changed, presence of residual chlorine, and dilutions to be used must be recorded.
- 14.2.8 Check and record the pH of each sample. The pH must be between 6.0 and 8.5. If it is not, adjust the pH by using 10% sulfuric acid (to lower the pH) or 10N sodium hydroxide (to raise the pH). If pH adjustment is expected to require more than 5 ml of reagent per 1 liter of sample, more concentrated reagents must be used. Record initial pH and adjusted pH of the sample into the sample logbook. Also record amount of chemical used to adjust pH and the standard ID. If dilutions are made, check the final pH.
- 14.2.9 Check the sample for residual chlorine by using the chlorine test tablet (Lamotte DPD #4). Add one tablet to a 13 x 100 mm test tube of sample. If sample turns bright pink within two minutes, chlorine is present. For each sample, record result with a "+" or "-" to indicate the presence or absence of chlorine. It is rare for a UBOD sample to be chlorinated, but it is possible if the sample was collected near an outfall.
- 14.2.10 If chlorine is present, dechlorinate sample following procedure. See Appendix F.
- 14.2.11 Pour the NO₃NO₂ sample for each UBOD sample/sample dilution into the labeled bottles. Acidify each NO₃NO₂ sample to a pH <2 with 10 % H₂SO₄ by adding 2 drops. Randomly check 10% of the bottles using pH paper to verify proper preservation. Place these bottles in the refrigerator. **The NO₃NO₂ samples must be analyzed within 28 days from the date of collection on reading days.**
- 14.2.12 Prepare GGA standards according to directions in [Section 10.3](#) using the aerated "nutrient" dilution water. Fill the 2 L BOD bottles. Pour the remaining standard volume into the glucose reservoir bottles.
- 14.2.13 Fill the "nutrient" dilution water blank bottles and their reservoirs with aerated "nutrient" dilution water.

- 14.2.14 Fill a 2 L BOD bottle for each sample and pour remaining sample into its reservoir bottle.
- 14.2.15 UBOD spreadsheets –
- 14.2.15.1 Raw data sheet – See Figure A. Open the Q drive and choose the UBOD folder. Open the BLANKS folder. Choose reading blank.xls. This sheet must be modified with the current information. Enter the correct survey name, the correct reading dates, and the new sample and site numbers. Only eight entries will fit on a page, so you must copy and paste the name and dates onto additional pages. Print out the corrected data sheets on legal paper. The daily readings will be recorded by hand on these sheets. Save the information until it is copied to the final data sheets.
- 14.2.15.2 Final data sheet – See Figure B. Open the Q drive and choose the UBOD folder. Open the BLANKS folder. Choose blank UBOD Survey x.xls. Copy the information from the raw data sheet. Save the file into the folder for the current year under the name of the new survey that is being set up. All readings must be manually transferred to this file. The depletion values will automatically be calculated. Enter the calculated values in the LIMS. See Section 14.5 for instructions on completing this spreadsheet.
- 14.2.16 After the samples have been warmed to 20°, checked for pH and chlorine, and adjusted, the D.O. can now be read. With a calibrated meter, determine the D.O. Record the value on the raw data sheet. Make a note of the time readings began. If the DO is at least 7.0 mg/L, pour off a 30mL NO₃NO₂ sample. Refill and stopper the bottle, making a watertight, airtight seal. Place a plastic cap on top of that. Put the samples and standards, in reading order, in the incubator along with their reservoirs. If sample has an initial DO < 5, the sample must be setup straight and with dilutions. See Section 14.2.21 for dilution instructions. If the sample D.O. is over the saturation level for the sample's temperature see Section 14.2.19.
- 14.2.17 If a sample needs to be aerated, follow these instructions:
- 14.2.17.1 Pour off some of the sample (approx. 100 ml) into a cup to prevent the sample from overflowing while it is being aerated. (Add this back to the bottle after aeration).
- 14.2.17.2 Place a clean disposable pipette on the end of the Tygon® tubing connected to an air cylinder (Ultra-pure Zero air) or "house air".
- 14.2.17.3 Place the pipette into the bottle and turn the "house air" or a cylinder of ultra pure zero air on. Adjust the airflow by turning the flow regulator to about 25 psi and allow the sample to aerate for 2 minutes or longer depending on the D.O. and volume of sample. Aeration time varies with each sample and air pressure, so a good rule of thumb is to subtract the current D.O. from 8.0. The result is how many minutes to aerate the sample, e.g. a sample has a DO of 5.0, so aerate the sample for 3.0 minutes. This method is not necessary, but it is a good rule of thumb.

- 14.2.17.4 After aeration, refill the bottle back up to the neck. Let the sample sit for a few minutes to eliminate any bubbles. Check sample DO with the calibrated meter to see if DO is near 8.0. If it is not, aerate a little longer to get the DO near 8.0 or higher, but do not exceed saturation level. (See O₂ Solubility Chart, Table 1 in [Section 23](#)).
- 14.2.18 After all aeration has been done, read the D.O. of the sample and record the final D.O. before the sample is stored until the next reading.
- 14.2.19 If the sample D.O. is over the saturation level for the sample's temperature (See O₂ Solubility Chart, [Table 1](#) in Section 23), pour the sample from the prepared BOD bottle into a 3000 ml beaker. Aerate for three minutes. Pour the sample back into the BOD bottle and read the new D.O. If the sample D.O. is still too high, repeat the aeration procedure. Be sure to record the original D.O. level and the value after final aeration.
- 14.2.20 Check the meter's calibration every 10 bottles by reading the D.O. of the calibration bottle. The meter must not drift more than 0.2 mg/L from the original calibration value. If so, discontinue reading samples and recalibrate the meter.
- 14.2.21 UBOD samples are set up undiluted, if possible. It is better to monitor D.O. levels more often than to set up diluted samples. If, after reviewing the chains of custody, rapid D.O. depletion and high UBOD are expected, set up applicable dilutions. If samples arrive at the lab with a D.O. of 5.0 or less, make dilutions of the sample and take a readings (usually DF: 4 and DF:10). (Dilutions are made with "**aerated**" DI water with no nutrients. DF:4 is 750 ml of sample to 3L of "**aerated**" DI water with no nutrients. DF:10 is 300 mL of sample to 3L of "**aerated**" DI water with no nutrients.) After a dilution is made with the original sample, take a reading of the original sample and then aerate it to a D.O. of 7 or above and then take another reading after the aeration. The original sample and its dilutions will be kept and read until it depletes, possibly before the end of the survey. Once the original sample depletes it may be discarded if a dilution had been made for that sample. If a dilution is not made on a sample and the sample depletes, a dilution must be made of the depleted sample and continued readings are done. Samples with a depletion per day of >1.5mg/L require dilutions. Sample types that may require dilutions are those that contain high concentrations of organic matter, have an odor, are dark, have a low field D.O. and/or pH, or have a facility name. Use the "**Aerated**" water to make dilutions for samples. The usual dilutions are DF: 4 and DF:10. However, if the sample comes from a facility that deals with animal matter (for example: seafood plant, chicken or alligator farm), a DF:100 may be needed. Set up an undiluted sample as well, even though it may deplete below 2.0 mg/L before the first reading. Prepare 3L of the diluted sample to allow enough for the reservoir. If a sample dilution was prepared with new "**Aerated**" dilution water; make a blank for diluted samples.

14.3 Reading Days

- 14.3.1 After Day 0, sample DO readings are usually taken on days 1, 4, 7, 11, 15, 20, 29, 40, 50 and 60. These days will vary with each survey's collection date and sample depletion rates. Additional readings may be taken if needed.
- 14.3.2 Aerate about 3 L of reagent water for 5 minutes. Use this water to calibrate the DO meter. Make sure the DO does not exceed that allowed for the temperature as shown on the oxygen solubility chart, [Section 23, Table 1](#).
- 14.3.3 Record on raw data sheet each reading's information: date, day number, analyst's initials, time, and the DO reading for each sample bottle and QC. On appropriate spreadsheet in the computer, enter for each day you read a survey the date, day number, analyst's initials, time and the DO reading for each QC or sample bottle.
- 14.3.4 Remove the samples from the incubator. First, remove the plastic cap, and invert the bottle while pressing on the stopper. This pours off the water seal around the stopper. Mix the sample by inverting the bottle. Rinse and insert the probe into the first bottle (the blank). Turn on the stirrer. The temperature of the sample must be between 19°C and 21 °C when reading DO.
- 14.3.5 If any air bubbles are seen in the bottle or around the membrane, turn stirrer off and partially lift the probe from the bottle to allow the bubbles to flow to the top of the bottle. Insert the probe into the bottle again and begin reading.
- 14.3.6 Wait for a stable reading (a beep will indicate a stable reading). Record the D.O. reading in the top cell of the spreadsheet for that sample and that reading number. Remove probe, rinse, and place it in the next sample or back in the 300 ml bottle that is half filled with water.
- 14.3.7 On readings after Day 0 if the D.O. of the laboratory reagent "BLANK" is higher than the previous reading by greater than 0.1, check the air calibration. Recheck meter calibration, if necessary, by repeating Winkler titration before reading samples. See Appendix D - Troubleshooting for more information.
- 14.3.8 After reading the DO, a nitrate-nitrite sample must be collected for every sample each day you read the survey (blanks and glucose do not need NO₃NO₂ analysis). Pour off a small volume of sample into a 15 - 30 ml Nalgene® bottle for NO₃NO₂ analysis. Label bottles according to section 14.2.11. Preserve the NO₃NO₂ sample to pH <2 with 10 % sulfuric acid (2 drops). Check 10% of the bottles with pH paper to confirm. Place these bottles in the refrigerator. **Make sure the nitrate-nitrite sample is pulled from the BOD bottle before any refilling or re-aeration is done.**
- 14.3.9 Sample DO levels must be kept above 2.0 mg/L. If the sample DO is expected to deplete below 2 before the next scheduled reading, the samples may be read again sooner or the sample may be re-aerated.

Determine whether the sample needs re-aeration by calculating the current rate of depletion (depletion / day), and using that to estimate the next reading. If the sample needs re-aeration, follow the instructions in Section 10.2.2.

- 14.3.9.1 If a sample's DO depletes rapidly between readings set up a dilution for that sample. Rapid depletion is > 1.5 mg/L per day. If the sample DO falls below 2 mg/L, then check the DO of the sample reservoir. If the DO of the reservoir is >2 mg/L, then set up a dilution using the sample's reservoir. Set up a reservoir bottle for the dilution as well. Also, set up a dilution blank (check blank) on the same day. If possible, keep the original sample and try to keep re-aerating it throughout the survey. Refer to Section 18.2.1.
 - 14.3.10 After collecting the NO_3NO_2 sample (or after re-aerating, if necessary), refill the 2L bottle to the neck from the sample's reservoir. Mix by inverting the bottle several times, then reread and record the DO of each sample into the bottom cell of the spreadsheet for the corresponding sample and reading number. Make sure the probe's stirrer is on. Remove the probe and rinse with reagent water. Gently shake any excess water off the probe and insert it into the next sample or into the half-filled 300 ml bottle. Place the ground-glass stopper in each bottle, making sure to make an airtight water seal. Place the plastic cap on top of the bottle. Put the sample bottle and its reservoir back into the incubator until the next reading.
 - 14.3.11 Check the meter's calibration every 10 bottles by reading the DO of the calibration bottle. The meter must not drift more than ± 0.2 mg/L from the original calibration value within 30 minutes. If so, discontinue reading samples and recalibrate the meter.
- 14.4 Last Reading Day (Day 60)
- 14.4.1 Follow the same instructions as the regular reading days, except additional parameters must be analyzed on the last day. The final sample pH becomes pH60 and must be batched. The pH60 must be measured and recorded in LIMS, along with QC standards for pH (See Appendix C). The following samples must be pulled from each UBOD sample bottle after the final DO is read:
 - 14.4.1.1 One hundred twenty-five (125) mL preserved to pH <2 with 10 % H_2SO_4 for TKN analysis (TKN60).
 - 14.4.1.2 A separate 125 ml is collected for TOC (TOC60) and preserved to pH <2 with 10 % HCl.
 - 14.4.1.3 A separate 30 ml is collected for NO_3NO_2 (UNN60) and preserved to pH <2 with 10% H_2SO_4 .
 - 14.4.1.4 Put the bottles in the sample refrigerator.
 - 14.4.2 After you finish the survey, dump the sample and reservoir down the sink with running tap water.

- 14.4.3 Place dirty BOD bottles in dishwasher and wash according to instructions in Appendix E.
- 14.5 Final data sheet –
- 14.5.1 Each sample has its own row. The top cell for each reading is the first DO reading. You will see the UBOD result for that reading appear. The bottom cell for the same reading number is for the DO after the Nitrate-Nitrite sample has been poured off and the sample has been refilled from the reservoir and/or re-aerated. This “final” reading will be used as the “initial” reading in the calculation of the next reading’s UBOD.
- 14.5.2 The calculations for the blanks and GGAs are always calculated from day zero to the current date, whereas the samples are calculated on a reading-to-reading basis. This is because only one reading per day is required for the QC samples. Blanks and GGAs are not altered by aeration or pouring off nitrate samples.
- 14.5.3 All samples will be calculated as UNDILUTED. If a sample has to be diluted, you must manually change the formula for EVERY UBOD calculation. See Section 15.6.2.
- 14.5.4 The glucose is also considered a dilution, but it is calculated differently than a sample because only one reading per day is taken for glucose. The dilution factor for glucose is 2000 ml / 40 ml, which is 50, and the blank correction is 0.98, since 1960 ml/2000 ml is blank water. See Section 15.5.3.
- 14.5.5 It is important that you do not skip any columns on the spreadsheet. If necessary, change the reading numbers and delete columns only from the very end.
- 14.6 Routine Maintenance
- 14.6.1 The probe membrane must be changed at least every two weeks or sooner if unstable readings are acquired, or there is obvious damage to the membrane. The probe must be cleaned only when one or more of the following conditions occur.
- When sporadic readings occur
 - When changing the membrane did not fix the problem.
 - When about 500 hours of use (about 2 months) have elapsed.
- 14.6.2 Excessive cleaning must be avoided since it reduces the life of the probe. All probe maintenance must be recorded in the BOD logbook.
- 14.6.3 Change the membrane/clean the probe as follows:
- 14.6.3.1 Turn off the meter.
- 14.6.3.2 Remove the stirrer from the probe by pulling downward.
- 14.6.3.3 Unscrew the old membrane cap from the probe.
- 14.6.3.4 Inspect the probe tip. If it is very discolored (dark) and/or there are signs of precipitate formation, follow each step below. If it is slightly

discolored, skip ahead to step 14.10.3.9 and only change the membrane.

- 14.6.3.5 Prepare 14% NH₄OH in a BOD bottle. In a hood, add 42 ml of NH₄OH to the 300-ml bottle and fill with reagent water. Mix the solution. Place the probe in this solution for about 3 minutes. Wipe off the probe with a tissue. If there is still dark coloration, repeat the process.
 - 14.6.3.6 Rinse the probe well and blot dry.
 - 14.6.3.7 Polish the gold tip of the probe with the sanding disc provided with the membrane caps. Gently sand the tip 2-3 times.
 - 14.6.3.8 Rinse the probe well and blot dry.
 - 14.6.3.9 Get a new membrane cap and fill it ½ full with the electrolyte solution provided in the box of membranes. Screw the cap onto the probe finger tight. It is normal for some of the electrolyte solution to overflow. Make sure there are no air bubbles trapped inside the membrane cap. If so, remove the membrane cap and repeat.
 - 14.6.3.10 Rinse off any excess electrolyte solution with reagent water. Attach the stirrer. .
 - 14.6.3.11 Place the probe in a 300 ml BOD bottle filled with reagent water.
 - 14.6.3.12 Turn on the stirrer and allow the probe to stabilize before proceeding with measurements. It takes at least 2 hours if just the membrane was changed and overnight if the probe was also cleaned.
- 14.6.4 Change the tubing connected to the purified air cylinder at least bimonthly or sooner if it becomes contaminated or discolored.

15.0 [Evaluation of Data, Reporting Results and Calculations](#)

- 15.1 All data must be evaluated before reporting. All quality control samples must be within designated control limits.
 - 15.1.1 All blanks must be less than 1.0 ppm at the end of 60 days.
 - 15.1.2 All LCSs must be within 300 mg/L +/- 30.5 at the end of 60 days.
- 15.2 All data must be entered with the designated decimal places, which is 1 decimal place for final results <100 and whole numbers for final results >100.
- 15.3 Samples are reported as total depletion of DO in mg /L.
- 15.4 Samples, field blanks, and blanks must be reported as their numeric value (no negative numbers).
- 15.5 If a dilution is used, enter the dilution factor into the analysis special info screen in LIMS.
- 15.6 The Excel spreadsheet automatically performs all calculations of UBOD when DO readings are entered. These calculations are accepted, but if manual calculations are necessary, or to verify the calculations in the spreadsheet, follow the calculations in the sections below.

- 15.6.1 Calculation for UBOD for Undiluted Sample, in mg/L = $(D_2 - D_1) +$
Previous UBOD

where D_2 = Final DO reading from previous day's reading, in mg/L

D_1 = Current DO reading, in mg/L

Previous UBOD = Total depletion to date, in mg/L.

- 15.6.2 Calculation for UBOD for diluted sample. Make sure to do calculation in the correct order of mathematical operations—parentheses first, then multiplication / division, and finally addition / subtraction.

$\{(D_2 - D_1) - (BTD) * \% \text{ Check Blank}\} * \text{Dilution Factor} + \text{Previous UBOD}$

where: D = Sample DO Readings in mg/L, 2 is the final reading from the previous reading day, 1 is the current reading

BTD = the blank's total depletion to date (current blank UBOD) in mg/L

$\% \text{ Check Blank}$ = percent of the total volume that is blank water

Dilution Factor, in ml = $(\text{total volume})/(\text{sample volume})$

- 15.6.3 GGA calculation is as follows:

$\{(G_0 - G_1) - (BTD * 0.98)\} * 50$

where G_0 = the initial DO reading for glucose (day 0) in mg/L,
 G_1 = current glucose DO reading in mg/L,

BTD = the blank's total depletion to date (current blank UBOD) in mg/L

- 15.6.4 Blanks are calculated as $D_0 - D_C$

where D_0 = Day zero DO reading, in mg/L

D_C = current day's DO reading, in mg/L.

There is no correction for the blank unless the sample is diluted.

- 15.7 Two Winkler titrations are done and the results are averaged. The difference in the values of both bottles must be within the range of +/- 0.2 mg/L. The average is the set point for the meter's calibration.
- 15.8 All raw data must be recorded in an Excel spreadsheet specific for each survey. When the survey is finished, the raw data is printed out, dated, and signed by the analyst. The raw data includes results for samples and quality control samples.
- 15.9 Results for the glucose-glutamic acid standard are entered into the Q drive UBOD>>year folder>>survey name. There is also a graph and spreadsheet which compares surveys. Those results are entered into the Q drive>>UBOD>>LCS>>year folder.

16.0 [Method Performance](#) [Link to Section 3](#)

16.1 All demonstrations of capability (accuracy and precision) must be determined at least annually or whenever there is a significant change in background or instrument response or when a new operator begins work.

16.1.1 Determination of Accuracy and Precision

Accuracy is measured by percent recovery and must be within control limits of the analysis. Percent recovery is calculated as follows:

$$PR = (C/TC)*100$$

Where: C = the concentration of the replicate and
TC = the known concentration of the sample.

The precision is measured by the calculating the standard deviation of the four samples. The standard deviation must be within designated control limits (20%).

16.1.1.1 To verify accuracy and precision of the method analyze four (4) replicate samples of a GGA. The accuracy must be within the limits of 300 mg/L \pm 30.5. The method's accuracy and precision shall be verified at least annually.

DOCs shall be determined when a new operator begins work, or whenever there is a significant change in background or instrument response.

16.1.2 Two blanks must be analyzed along with the 4 GGAs for calculation purposes.

16.1.3 Documentation of the initial DOC must be kept in the analyst's training file. Training files are kept by the section supervisor.

16.2 Continuing Demonstration of Capability

16.2.1 Once initial laboratory and analyst capability are verified and established, a program of continued verification of data quality must be maintained. This requires:

16.2.1.1 Ongoing analysis of blanks and GGAs as described in Section 12.

16.2.1.2 Regular calibration and maintenance of laboratory instrumentation.

16.2.1.3 Maintenance of quality control records and charts.

16.2.2 Annual performance of at least one of the following is required:

16.2.2.1 Analysis of four consecutive LCSs with acceptable recovery and precision, or

16.2.2.2 An initial DOC study.

17.0 [Pollution Prevention](#)

- 17.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation.
- 17.2 The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.
- 17.3 BOD samples may be discarded in a lab sink.

18.0 [Data Assessment and Acceptance Criteria](#)

- 18.1 Data shall be reported with no cautionary flags if the following criteria are met:
 - 18.1.1 The depletion of the blank in a batch must be less than 0.5 ppm DO in 20 days and 1.0 ppm DO in 60 days.
 - 18.1.2 Laboratory control samples (LCS) of a batch have values which are 300 mg/L +/- 30.5. See [Section 12](#) for more information.
 - 18.1.3 Sample D.O. does not read below 2.0 mg/L on any reading day.
- 18.2 Data that do not meet all of the above criteria may be reportable in the following cases with a flag and an explanatory narrative. Such cases must be reviewed by the Scientist Manager:
 - 18.2.1 If the D.O. in a bottle drops to < 2 mg/L, the UBOD results for that day, as well as all successive UBOD results, are reported in LIMS with the qualifier "J", to indicate that the actual value is greater than the result reported. The following comment must be added to the Analysis comment section: "D.O. depleted below 2.0 mg/L, so all subsequent readings are reported as greater than, with qualifier "J". " The Sample comment section must have the comment: "UBOD: On (enter date), the D.O. dropped below 2.0 mg/L, so subsequent readings are reported as greater than, with qualifier "J"."
- 18.3 Data collected with a sample that fails collection, storage, or holding time requirements are reported using a data qualifier of "J" when logged into the LIMS system. A narrative must be written to accompany the sample reports explaining the problem(s) with the sample(s).

19.0 [Corrective Action for Out of Control Data](#)

- 19.1 A corrective action must be taken if any of the following occurs:
 - 19.1.1 If a sample was not set up within the holding time, in this case 48 hours.
 - 19.1.2 If a sample has been reported with the incorrect result due to entry error.

20.0 [Contingencies for Handling Unacceptable Data](#)

- 20.1 An entire day's reading must be rejected if the blank is out of control. Add an additional reading day to substitute for this failed reading day. Regardless, consult with supervisor and/or manager.
- 20.2 If problems persist, see [Section 18](#) for reporting options.

21.0 [Waste Management](#)

- 21.1 Sample disposal depends on the concentration of the analyte. Samples with analyte concentrations below waste disposal standards (check reference) are disposed of in the sink, preferably in a hood, while rinsing with water. Samples with analyte concentrations above the waste disposal standards are disposed of in the organic waste disposal. For our purposes, all samples can be disposed of in the sink.
- 21.2 All unused samples and reservoirs are held to expiration, and are discarded in the same manner necessary for their analyte concentration.

22.0 [Data and Records Management](#)

- 22.1 Procedures for data and record management must adhere to the Quality Manual and subordinate documents covering record keeping and the document control plan. All records shall be stored in such a way as to be safe and accessible for at least 10 years. All activities reported in lab notebooks must be signed and dated by the analyst.
- 22.2 A binder containing all certificates of analysis of standards used must be kept up to date by the analyst. Certificates shall be marked with the receiving date.
- 22.3 A logbook containing the standards/samples prepared must be kept up to date by the analyst. This logbook is used to track the standard/samples prepared, the date prepared, preparation, name, and lot number and expiration date.
- 22.4 A logbook is kept for a record of all samples analyzed. Analyst’s signature and date are required.
- 22.5 A logbook is kept for the pH meter for calibrations. Also, all ongoing maintenance and preventative maintenance must be documented in the logbook and maintenance records.
- 22.6 A logbook is kept for the D.O. meter for calibrations. The D.O. meter logbook is kept indicating the temperature of the water used for “Winkler Titration” along with the results of “Winkler Titration” and the reagents used for the titration.
- 22.7 Copies of the D.O. bench sheet, raw data for samples and quality controls samples and logbooks are stored in a centrally located file cabinet in Room B01 until archived.

23.0 [Tables, Diagrams, Flowcharts, and Validation Data](#)

Table 1: OXYGEN SOLUBILITY AND CALIBRATION TABLE
Solubility of Oxygen in mg/L in Water Exposed to Water Saturated Air 760 mm Hg Pressure.

TEMP °C	Chlorinity: 0	5.0	10.0	15.0	20.0	25.0
	Salinity: 0	9.0	18.1	27.1	36.1	45.2
18.0	9.47	8.97	8.50	8.05	7.62	7.22
19.0	9.28	8.79	8.33	7.90	7.48	7.09

20.0	9.09	8.62	8.17	7.75	7.35	6.96
21.0	8.92	8.46	8.02	7.61	7.21	6.84
22.0	8.74	8.30	7.87	7.47	7.09	6.72
23.0	8.58	8.14	7.73	7.34	6.96	6.61
24.0	8.42	7.99	7.59	7.21	6.84	6.50
25.0	8.26	7.85	7.46	7.08	6.73	6.39
26.0	8.11	7.71	7.33	6.96	6.62	6.29
27.0	7.97	7.58	7.20	6.85	6.51	6.18
28.0	7.83	7.44	7.08	6.73	6.40	6.09
29.0	7.69	7.32	6.96	6.62	6.30	5.99

Figure A: Raw data sheet

The screenshot shows a Microsoft Excel window titled "Microsoft Excel - Blank BOD Surveyx". The spreadsheet contains a template for BOD surveys. The columns are labeled A through J. The data rows (9-18) show readings for two blank samples and two sample runs (#1 and #2), each with Glucose and Glutamic Acid. The UBOD1, UBOD2, and UBOD3 values are all 0. The cell C13 is selected, showing the formula bar with an equals sign.

	A	B	C	D	E	F	G	H	I	J
1										
2										
3										
4	Incubator#:	Reading 0	Reading 1	UBOD1	Reading 2	UBOD2	Reading 3	UBOD3	Reading 4	
5		Day#:	Day#:		Day#:		Day#:		Day#:	
6		Date:	Date:		Date:		Date:		Date:	
7	Sample Number	Time:	Time:		Time:		Time:		Time:	
8	Site Number	Analyst:	Analyst:		Analyst:		Analyst:		Analyst:	
9	BLANK #1			0		0		0		
10				0		0		0		
11	BLANK #2			0		0		0		
12				0		0		0		
13	# 1 - Glucose-			0		0		0		
14	Glutamic Acid			0		0		0		
15	# 2 - Glucose-			0		0		0		
16	Glutamic Acid			0		0		0		
17				0		0		0		
18				0		0		0		

Figure B: Final data sheet

	A	B	C	D	E	F	G	H	I	J	K	L
1						(Survey Name) BOD Survey						
2												
3												
4	Incubator#:	Reading 0	Reading 1	Reading 2	Reading 3	Reading 4	Reading 5	Reading 6	Reading 7	Reading 8	Reading	
5		Day#:	Day#:	Day#:	Day#:	Day#:	Day#:	Day#:	Day#:	Day#:	Day#:	Day#:
6	Sample Number	Date:	Date:	Date:	Date:	Date:	Date:	Date:	Date:	Date:	Date:	Date:
7		Time:	Time:	Time:	Time:	Time:	Time:	Time:	Time:	Time:	Time:	Time:
8	Site Number	Analyst:	Analyst:	Analyst:	Analyst:	Analyst:	Analyst:	Analyst:	Analyst:	Analyst:	Analyst:	Analyst:
9	BLANK #1											
10												
11	BLANK #2											
12												
13	# 1 - GGA											
14												
15	# 2 - GGA											
16												

24.0 [References](#)

- 24.1 Standard Methods for the Examination of Water and Wastewater, 18th edition, 1992, pp. 5-3 – 5-6, Method 5210B.
- 24.2 Standard Methods for the Examination of Water and Wastewater, 19th edition, 1995, pp. 5-6 – 5-9, Method 5210C Proposed.
- 24.3 Standard Methods for the Examination of Water and Wastewater, 20th edition, 1998, pp. 5-7 – 5-9, Method 5210C Proposed.
- 24.4 YSI 5905/5010 BOD Probe Instruction Manual.
- 24.5 YSI model 5100 DO Meter Instruction Manual.

[Appendix A](#). Batching Samples Into LIMS and Entering Data

[Go to next Appendix](#)

A1.0 Logging on to the System

A1.1 Click on **Labworks** icon on the desktop.

A1.2 Enter user name and password.

A2.0 QA/QC Batching

A2.1 Each batch for UBOD is batched by using a study group of analysis relating to the UBOD analysis. This is designated by typing #UBOD. The UBOD study group includes all UBOD readings (1-60 day), all UNN (0-60 day) analyses, TKN60, TOC60, and pH60. All these test codes will appear when the batching procedure begins.

A2.2 To create new batches, click on the **QA/QC** icon or choose the **QA/QC** command from the **File** menu followed by **QA Batching**.

A2.3 Click on icon to batch sample by analyses.

A2.4 From the Sample Selection options click on **Unbatched samples with selected analyses pending**. (This option is for all samples with no data ever entered.) The **All samples with selected analyses pending** option is used for samples that have been previously batched.

A2.5 Select the appropriate analyses from the “Analyses available for batching” list by scrolling to the appropriate analyses or by typing the analyses in the text field. For UBOD, type in #UBOD.

A2.6 Click **OK**.

A2.7 Labworks performs a backlog search and generates a list of samples available for batching. This list will be displayed in the window labeled **Samples Selected for Batching**. Click **OK**.

A2.8 The batch selection screen appears and allows you to select the samples available. Decide which analysis to select. Do not select all of them. On setup day, choose UBOD60 for batching only. After each UBOD reading, you will batch that reading only. Deselect all other analysis by clicking off the red arrow next to Batch located below the analysis. Once the appropriate analysis has been chosen, click **OK**. See Figures 1 thru 4.

Batch Selections

Pending unbatched Analysis complete
 Pending batched Not ordered

OK Cancel

Samp ID	Location	U8BOD	U8NN	U9BOD	U9NN	UBOD60	UNN60
		<input checked="" type="checkbox"/> Batch					
AF01826	0447UBOD	<input checked="" type="checkbox"/> Pending		<input checked="" type="checkbox"/> Pending			
AF01831	0488UBOD	<input checked="" type="checkbox"/> Pending		<input checked="" type="checkbox"/> Pending			
AF01836	0488UBOD	<input checked="" type="checkbox"/> Pending		<input checked="" type="checkbox"/> Pending			
AF01841	FBUBOD	<input checked="" type="checkbox"/> Pending		<input checked="" type="checkbox"/> Pending			
AF01846	0489UBOD	<input checked="" type="checkbox"/> Pending		<input checked="" type="checkbox"/> Pending			
AF01851	0490UBOD	<input checked="" type="checkbox"/> Pending		<input checked="" type="checkbox"/> Pending			
AF01856	0491UBOD	<input checked="" type="checkbox"/> Pending		<input checked="" type="checkbox"/> Pending			
AF01861	0497UBOD	<input checked="" type="checkbox"/> Pending		<input checked="" type="checkbox"/> Pending			
AF04740	FBUBOD	<input checked="" type="checkbox"/> Pending					
AF04745	0450UBOD	<input checked="" type="checkbox"/> Pending					

Figure 1

Batch Selections

Pending unbatched Analysis complete
 Pending batched Not ordered

OK Cancel

Samp ID	Location	PH60	TKN60	TOC60	U0NN	U10NN	U1BOD
		<input type="checkbox"/> Batch					
AF01826	0447UBOD	<input type="checkbox"/> Pending	<input type="checkbox"/> Pending	<input type="checkbox"/> Pending		<input type="checkbox"/> Pending	<input type="checkbox"/> Pending
AF01831	0488UBOD	<input type="checkbox"/> Pending	<input type="checkbox"/> Pending	<input type="checkbox"/> Pending		<input type="checkbox"/> Pending	<input type="checkbox"/> Pending
AF01836	0488UBOD	<input type="checkbox"/> Pending	<input type="checkbox"/> Pending	<input type="checkbox"/> Pending		<input type="checkbox"/> Pending	<input type="checkbox"/> Pending
AF01841	FBUBOD	<input type="checkbox"/> Pending	<input type="checkbox"/> Pending	<input type="checkbox"/> Pending		<input type="checkbox"/> Pending	<input type="checkbox"/> Pending
AF01846	0489UBOD	<input type="checkbox"/> Pending	<input type="checkbox"/> Pending	<input type="checkbox"/> Pending		<input type="checkbox"/> Pending	<input type="checkbox"/> Pending
AF01851	0490UBOD	<input type="checkbox"/> Pending	<input type="checkbox"/> Pending	<input type="checkbox"/> Pending		<input type="checkbox"/> Pending	<input type="checkbox"/> Pending
AF01856	0491UBOD	<input type="checkbox"/> Pending	<input type="checkbox"/> Pending	<input type="checkbox"/> Pending		<input type="checkbox"/> Pending	<input type="checkbox"/> Pending
AF01861	0497UBOD	<input type="checkbox"/> Pending	<input type="checkbox"/> Pending	<input type="checkbox"/> Pending		<input type="checkbox"/> Pending	<input type="checkbox"/> Pending
AF04740	FBUBOD	<input type="checkbox"/> Pending					
AF04745	0450UBOD	<input type="checkbox"/> Pending					

Figure 2

Batch Selections

Pending unbatched Analysis complete
 Pending batched Not ordered

OK Cancel

Samp ID	Location	U1NN	U2BOD	U2NN	U3BOD	U3NN	U4BOD
		<input type="checkbox"/> Batch					
AF01826	0447UBOD		<input type="checkbox"/> Pending		<input type="checkbox"/> Pending		<input type="checkbox"/> Pending
AF01831	0488UBOD		<input type="checkbox"/> Pending		<input type="checkbox"/> Pending		<input type="checkbox"/> Pending
AF01836	0488UBOD		<input type="checkbox"/> Pending		<input type="checkbox"/> Pending		<input type="checkbox"/> Pending
AF01841	FBUBOD		<input type="checkbox"/> Pending		<input type="checkbox"/> Pending		<input type="checkbox"/> Pending
AF01846	0489UBOD		<input type="checkbox"/> Pending		<input type="checkbox"/> Pending		<input type="checkbox"/> Pending
AF01851	0490UBOD		<input type="checkbox"/> Pending		<input type="checkbox"/> Pending		<input type="checkbox"/> Pending
AF01856	0491UBOD		<input type="checkbox"/> Pending		<input type="checkbox"/> Pending		<input type="checkbox"/> Pending
AF01861	0497UBOD		<input type="checkbox"/> Pending		<input type="checkbox"/> Pending		<input type="checkbox"/> Pending
AF04740	FBUBOD	<input type="checkbox"/> Pending					
AF04745	0450UBOD	<input type="checkbox"/> Pending					

Figure 3

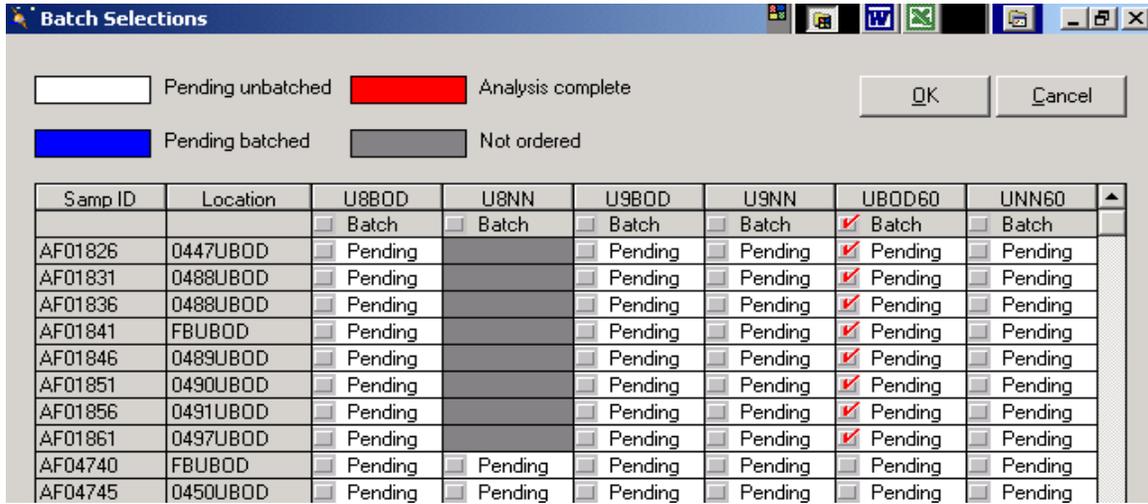


Figure 4

A2.9 In the **Batch Size Specification** screen the number of samples selected for batching as well as the batch size are shown. For batches less than 10 samples, only one batch is necessary. If more than 10 samples are in the survey, more batches will be created with appropriate QC. Each batch will only contain 10 samples. Click **OK**. See Figure 5.

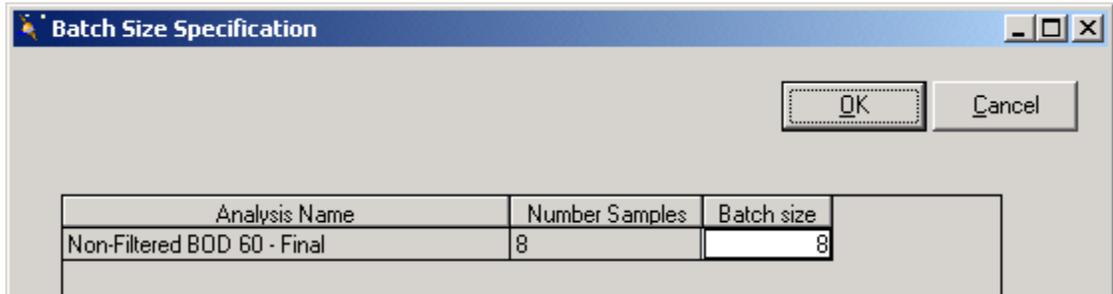


Figure 5

A2.10 In the **Batch QA Sample Specification** screen, the new batches that will be created are displayed. First, the user must clone each existing batch to reflect the number of QA tests to be run. Only the final reading of the survey needs to be cloned. Make four clones for the first batch. For surveys with > 10 samples, all other batches after the first batch will be cloned once for an LCS. Batch other readings with no QC added. Click **OK**. See Figure 6.

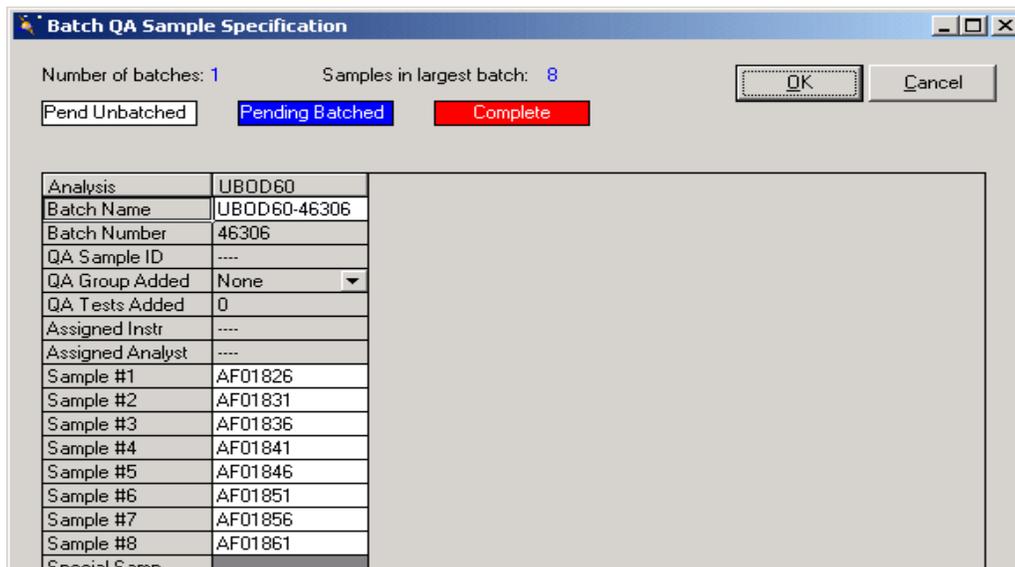


Figure 6

A2.11 Before cloning the original batch, enter in the appropriate data fields, the analyst's initials and the instrument used (YSI5100, a DO meter). These data fields will be carried through the entire cloning process. For instrument selection, click on the blank field next to Assigned Instr. When Instrument Selection screen appears, select appropriate instrument. Click **OK**. See Figure 7.

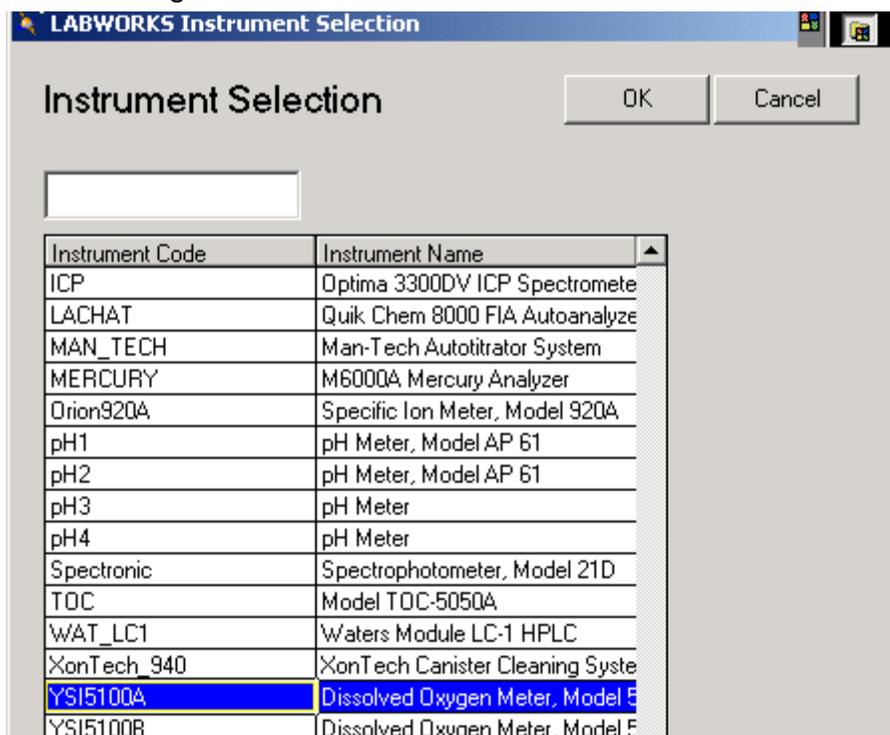


Figure 7

For analyst's initials, click on the blank field next to Assigned analyst.
When Analyst Selection screen appears, select appropriate analyst initials.
Click **OK**. See Figure 8.

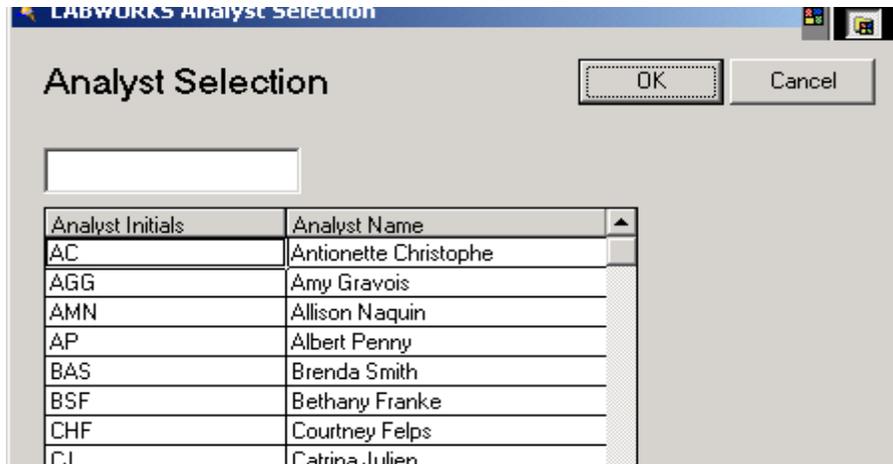


Figure 8

Once this is complete, cloning can begin. See Figure 9.

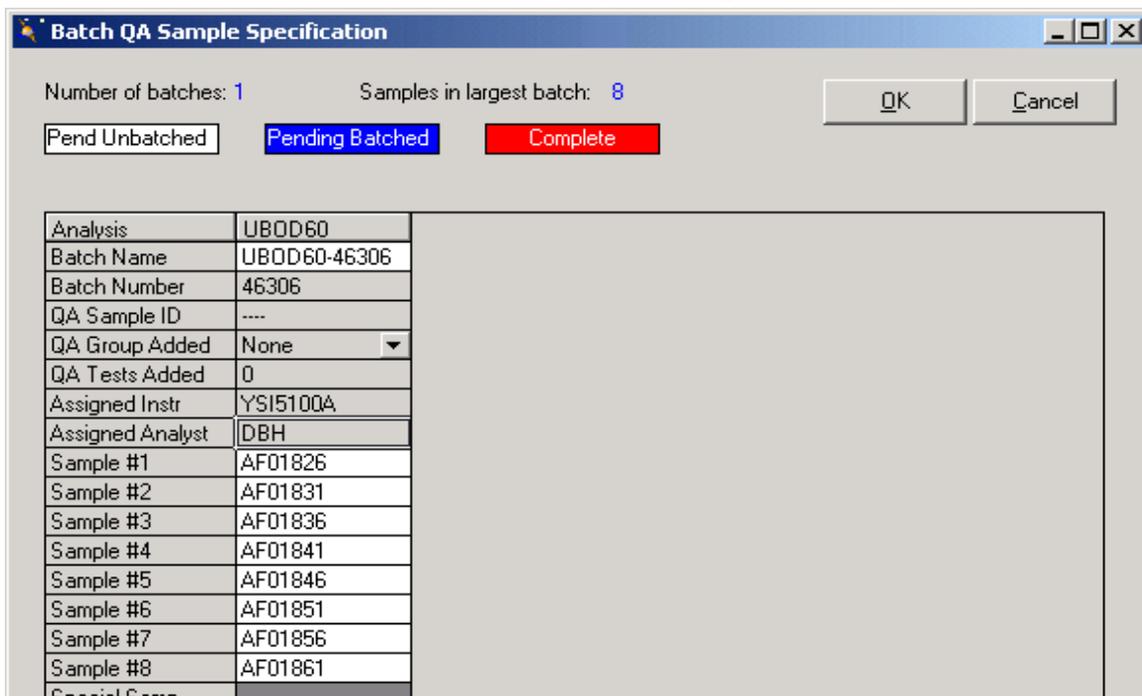


Figure 9

A2.12 Clone by right clicking on the batch header and select Clone Batch. The batch will be cloned and designated by the batch number #####-A. Clone the original batch again and the new batch will have the number #####-B. For the initial UBOD60 batch, 4 clones are necessary, two for blanks and two for LCS. See Figure 10.

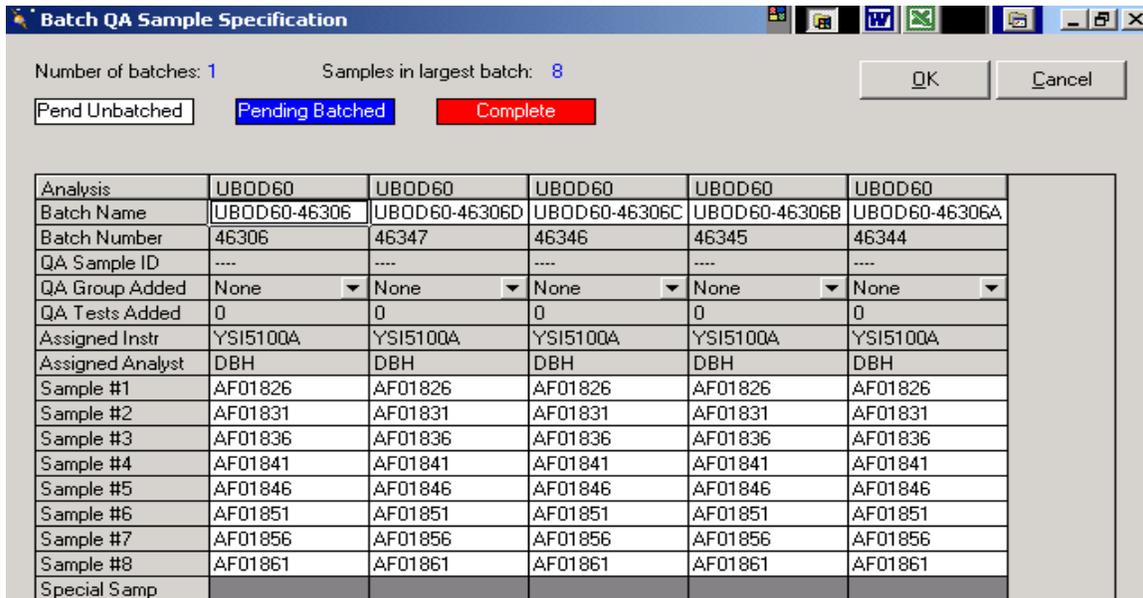


Figure 10

A2.13 Once the batch has been cloned the appropriate number of times the QA tests must be assigned to each batch. At the first cloned batch (#####A), now log in a QA sample. Right click on the empty cell after the last sample to bring up the menu to log in a QA sample. Select **QABLANK**. Click **OK**. See Figure 11.

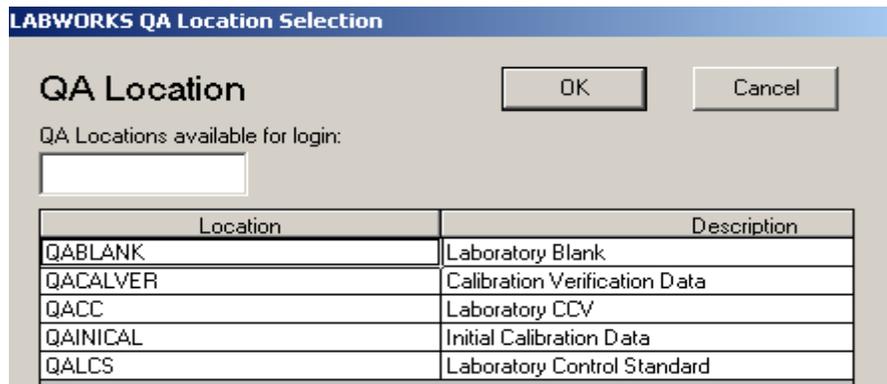


Figure 11

A2.14 At this point the system will log a QA sample into the system, give it a Labworks ID number, and place it in the batch. See Figure 12.

Batch QA Sample Specification

Number of batches: 1 Samples in largest batch: 8

Analysis	UBOD60	UBOD60	UBOD60	UBOD60	UBOD60
Batch Name	UBOD60-46306	UBOD60-46306D	UBOD60-46306C	UBOD60-46306B	UBOD60-46306A
Batch Number	46306	46347	46346	46345	46344
QA Sample ID	----	----	----	----	AF05716
QA Group Added	None	None	None	None	#QUBOD60
QA Tests Added	0	0	0	0	22
Assigned Instr	YSI5100A	YSI5100A	YSI5100A	YSI5100A	YSI5100A
Assigned Analyst	DBH	DBH	DBH	DBH	DBH
Sample #1	AF01826	AF01826	AF01826	AF01826	AF01826
Sample #2	AF01831	AF01831	AF01831	AF01831	AF01831
Sample #3	AF01836	AF01836	AF01836	AF01836	AF01836
Sample #4	AF01841	AF01841	AF01841	AF01841	AF01841
Sample #5	AF01846	AF01846	AF01846	AF01846	AF01846
Sample #6	AF01851	AF01851	AF01851	AF01851	AF01851
Sample #7	AF01856	AF01856	AF01856	AF01856	AF01856
Sample #8	AF01861	AF01861	AF01861	AF01861	AF01861
Special Samp					AF05716

Figure 12

A2.15 Now click on the box with the number 22 and select only the tests associated to the blank. All 22 QC tests associated with UBOD will appear. See Figure 13.

Analyses to Add to UBOD60 Sample AF05917

Special analyses for QA/QC sample:

- B_U1BUD - blank, Non-Filtered BOD 60 - Reading 1
- B_U2BUD - blank, Non-Filtered BOD 60 - Reading 2
- B_U3BUD - blank, Non-Filtered BOD 60 - Reading 3
- B_U4BUD - blank, Non-Filtered BOD 60 - Reading 4
- B_U5BUD - blank, Non-Filtered BOD 60 - Reading 5
- B_U6BUD - blank, Non-Filtered BOD 60 - Reading 6
- B_U7BUD - blank, Non-Filtered BOD 60 - Reading 7
- B_U8BUD - blank, Non-Filtered BOD 60 - Reading 8
- B_U9BUD - blank, Non-Filtered BOD 60 - Reading 9
- B_U10BUD - blank, Non-Filtered BOD 60 - Reading 10
- B_UBOD60 - Blank, Non-Filtered BOD 60 - Final
- L_U1BUD - GGA, Non-Filtered BOD 60 - Reading 1
- L_U2BUD - GGA, Non-Filtered BOD 60 - Reading 2
- L_U3BUD - GGA, Non-Filtered BOD 60 - Reading 3
- L_U4BUD - GGA, Non-Filtered BOD 60 - Reading 4
- L_U5BUD - GGA, Non-Filtered BOD 60 - Reading 5
- L_U6BUD - GGA, Non-Filtered BOD 60 - Reading 6
- L_U7BUD - GGA, Non-Filtered BOD 60 - Reading 7
- L_UBOD60 - GGA, Non-Filtered BOD 60 - Reading 8
- L_U9BUD - GGA, Non-Filtered BOD 60 - Reading 9
- L_U10BUD - GGA, Non-Filtered BOD 60 - Reading 10
- L_UBOD60 - GGA, Non-Filtered BOD 60 - Final

Figure 13

A2.16 Uncheck the arrows pertaining to the L_UBOD (GGA), leaving the 11 test codes for the blank. Click **OK**. See Figure 14.

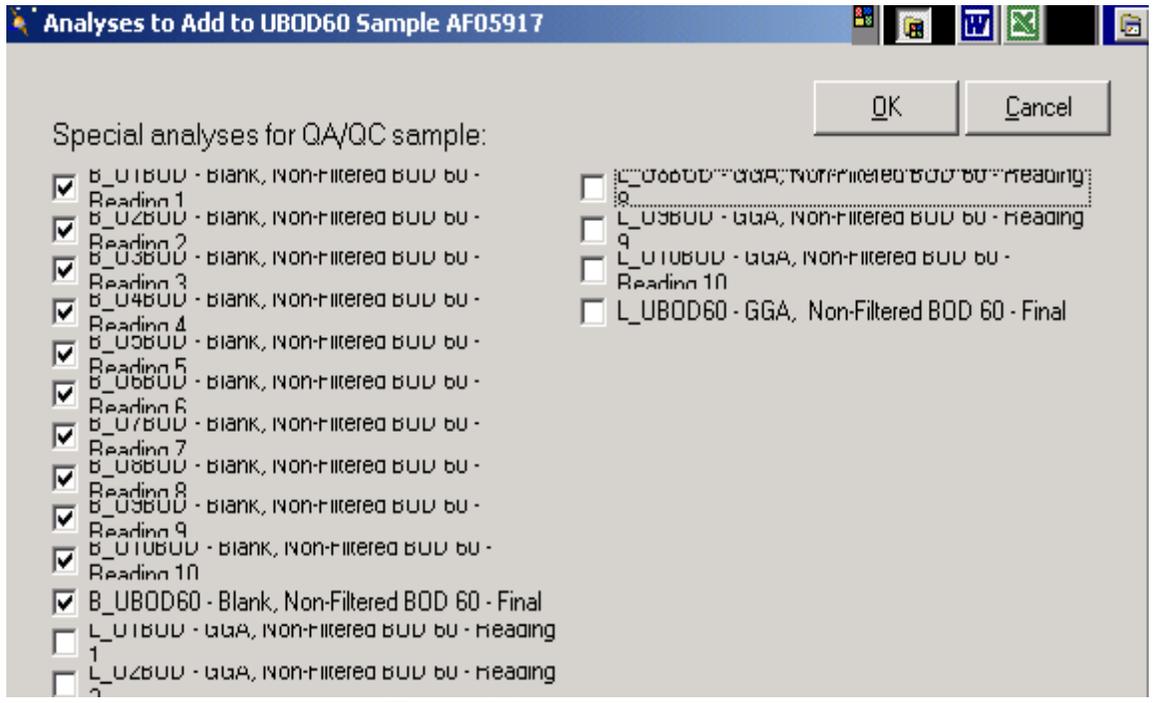


Figure 14

A2.17 Repeat steps 2.13 thru 2.16 for the second blank.

A.2.18 For the third clone, after logging in the QC sample, the test codes assigned will pertain to the LCS. See Figure 15.

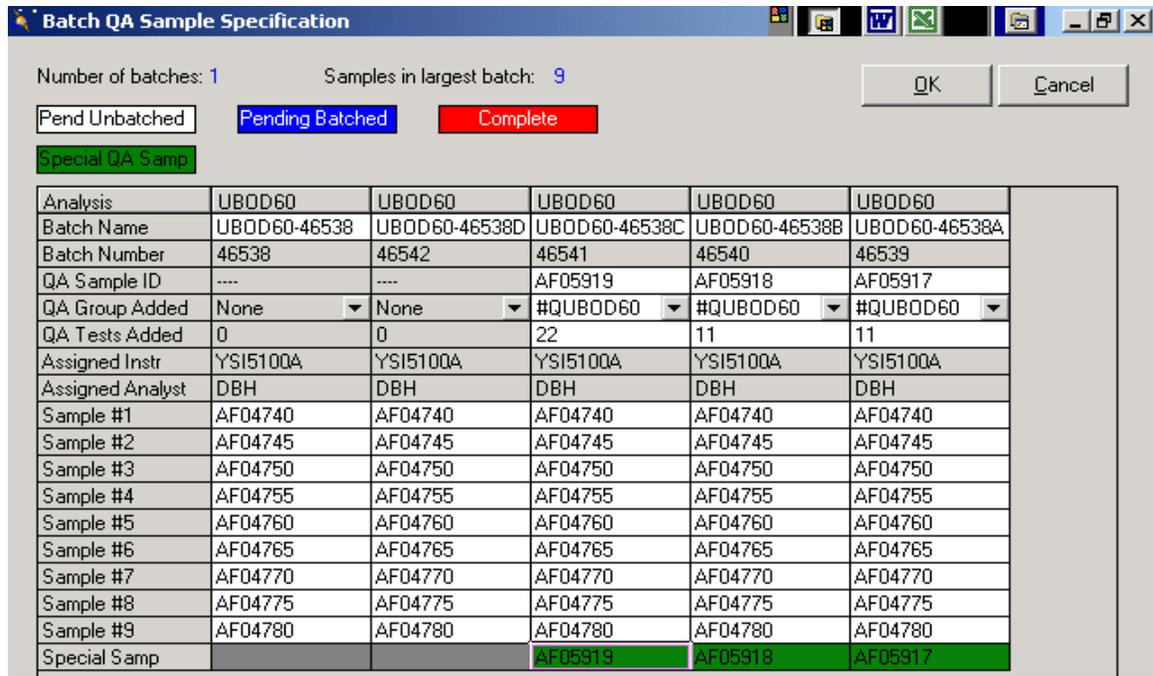


Figure 15

A2.19 In the QA location screen, choose **QALCS**. Click **OK**. See Figure 14.

A2.20 Now uncheck the arrows pertaining to the blank, leaving the 11 test codes for the LCS (GGA). Click **OK**. See Figure 16.

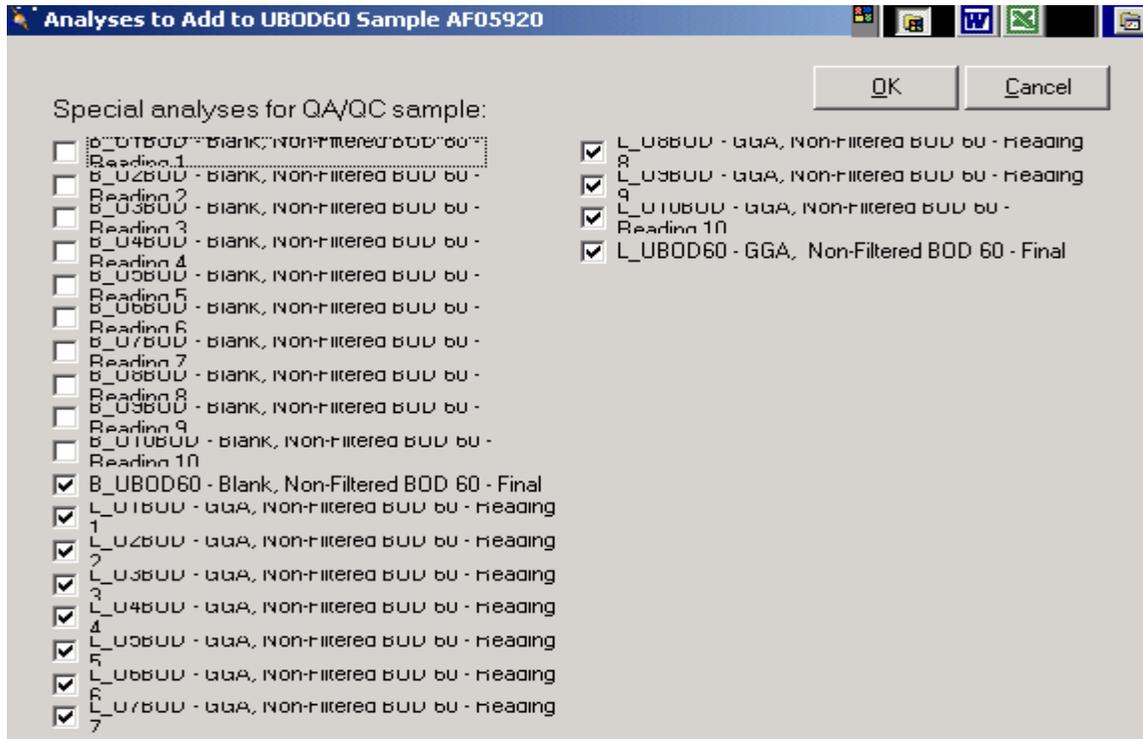


Figure 16

A2.21 Repeat steps 2.19 and 2.20 for the second LCS. When finished logging in all 4 QC samples and assigning test, click **OK** to finish creating the batches. See Figure 17.

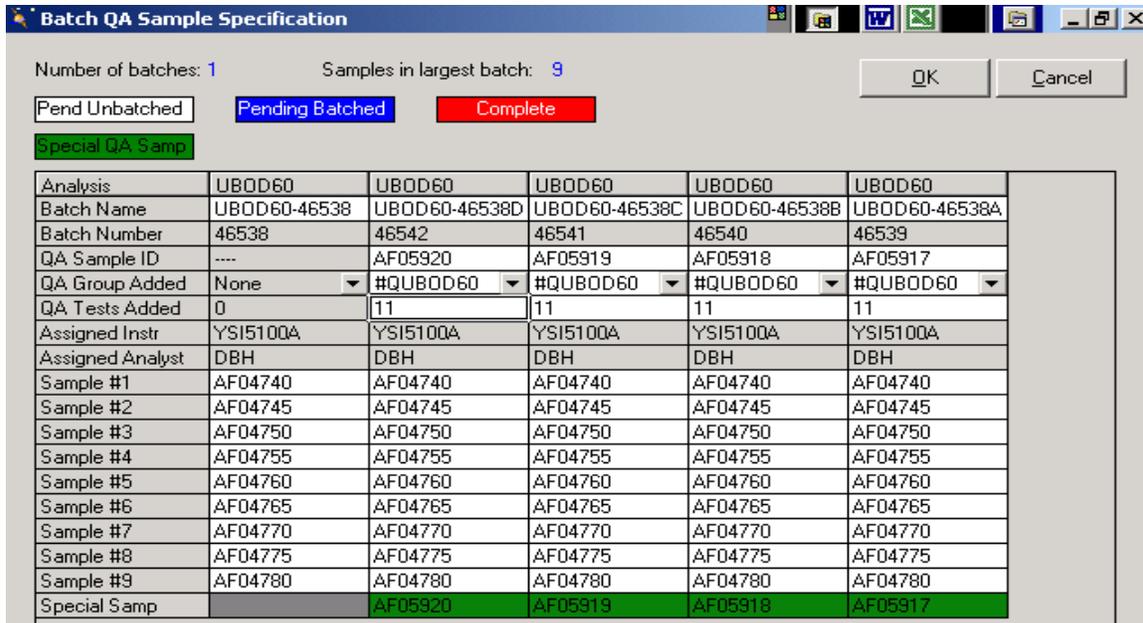


Figure 17

A2.22 Once the batches are created by LIMS, Figure 18 appears. Click **OK** to finish the batching procedure. See Figure 18.



Figure 18

A2.23 For surveys with > 10 samples, all other batches after the first batch will be cloned once for an LCS.

A2.24 For the individual UBOD readings follow the batching procedure except for the cloning of QC samples. Clone on the day the reading is taken and enter the data as in Section A3.0.

A3.0 Entering Data Into LIMS

A3.1 From the LABWORKS ES Desktop, Select **Results Entry** from the pull down menu on the **Results** tab. See Figure 19.

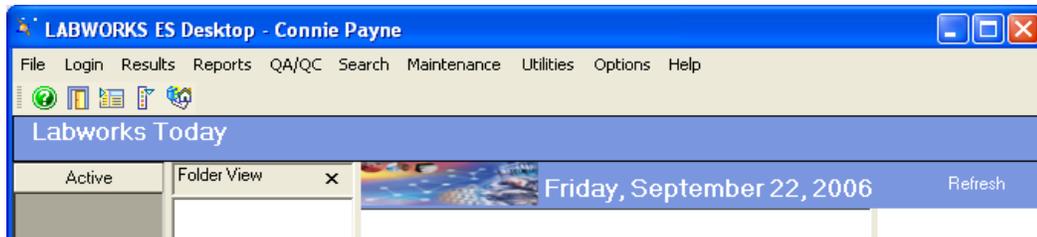


Figure 19

A3.2 On the **Results Sample Entry** screen, expand the choices under **QA/QC batch** folder. Open the **Batched Analyses** folder. See Figure 20.

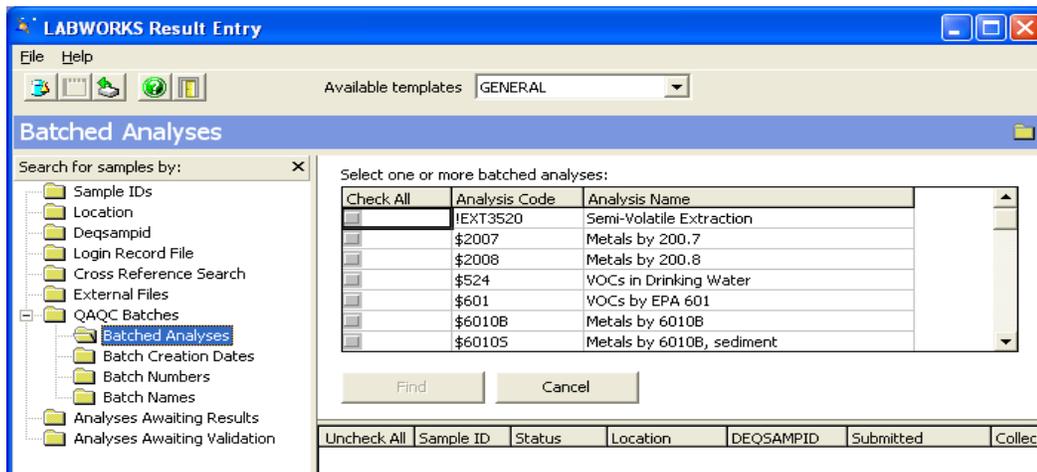


Figure 20

A3.3 The batched analyses appear to right on a scrolling menu. Click to select the analysis codes for each reading (i.e. U1BOD, U2BOD, etc.). When all selections have been made, click **Find**. See Figure 21.

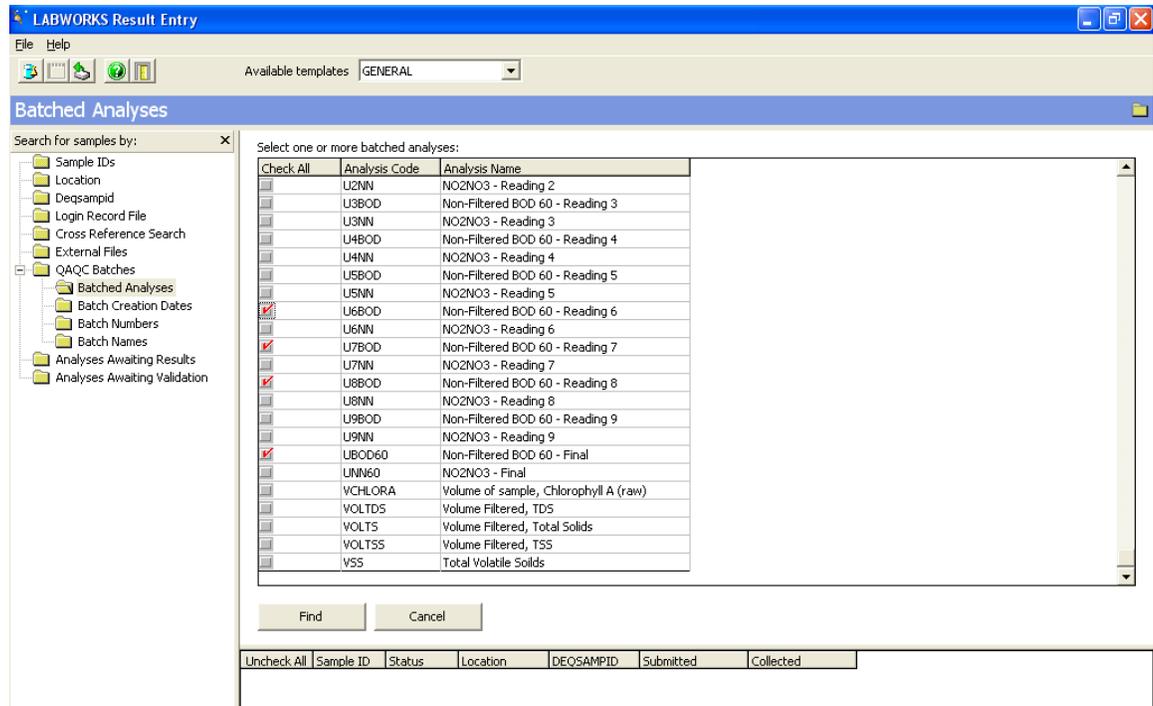


Figure 21

A pop-up will ask you if you want to query all samples. Choose **Yes**. See Figure 22.

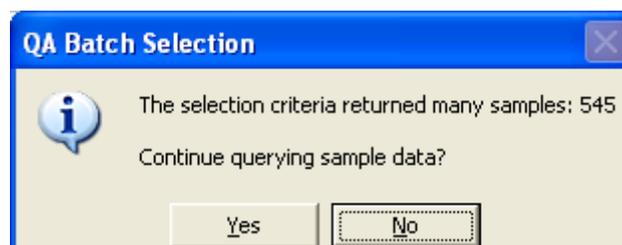


Figure 22

A3.4 Click on the **Date Created** column to choose analysis by date. See Figure 23.

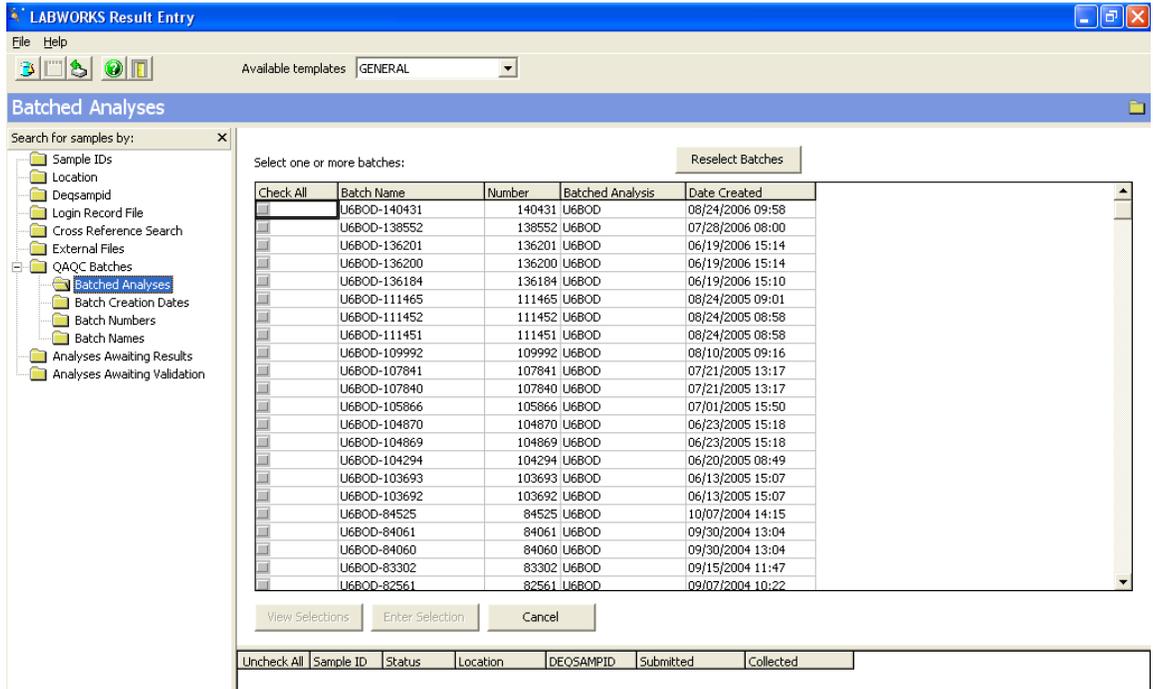


Figure 23

A3.5 The first click will arrange them in ascending order. See Figure 24.

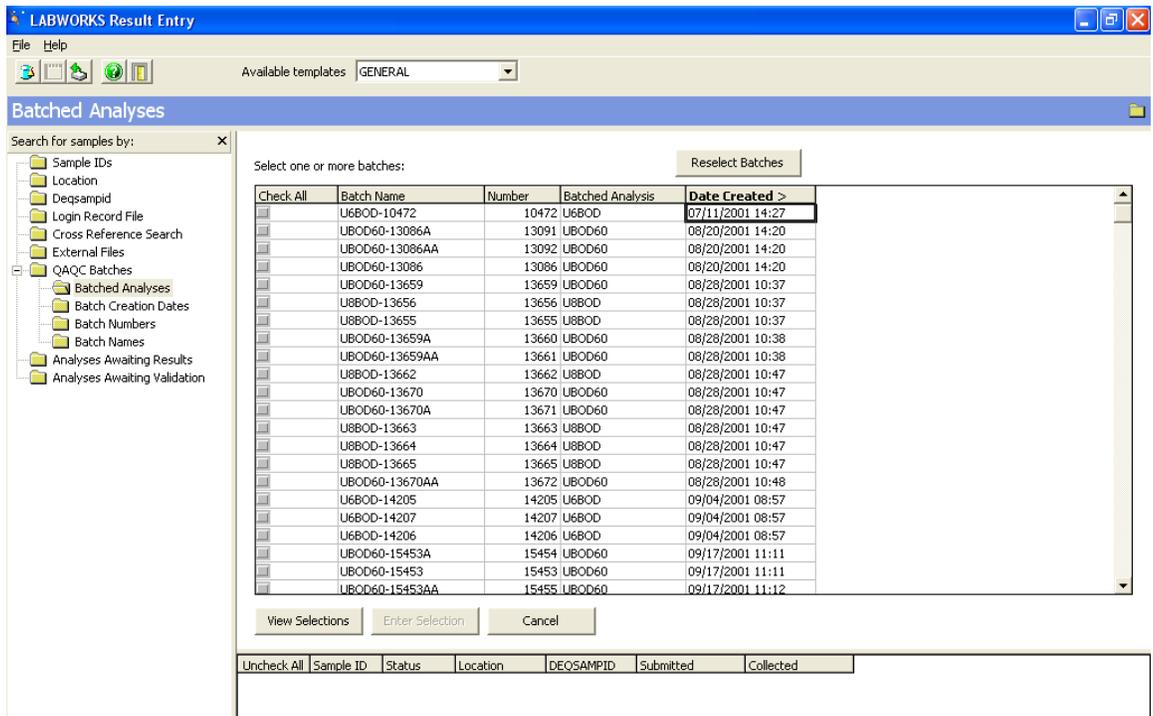


Figure 24

A3.6 The second click will arrange them in descending order. See Figure 25.

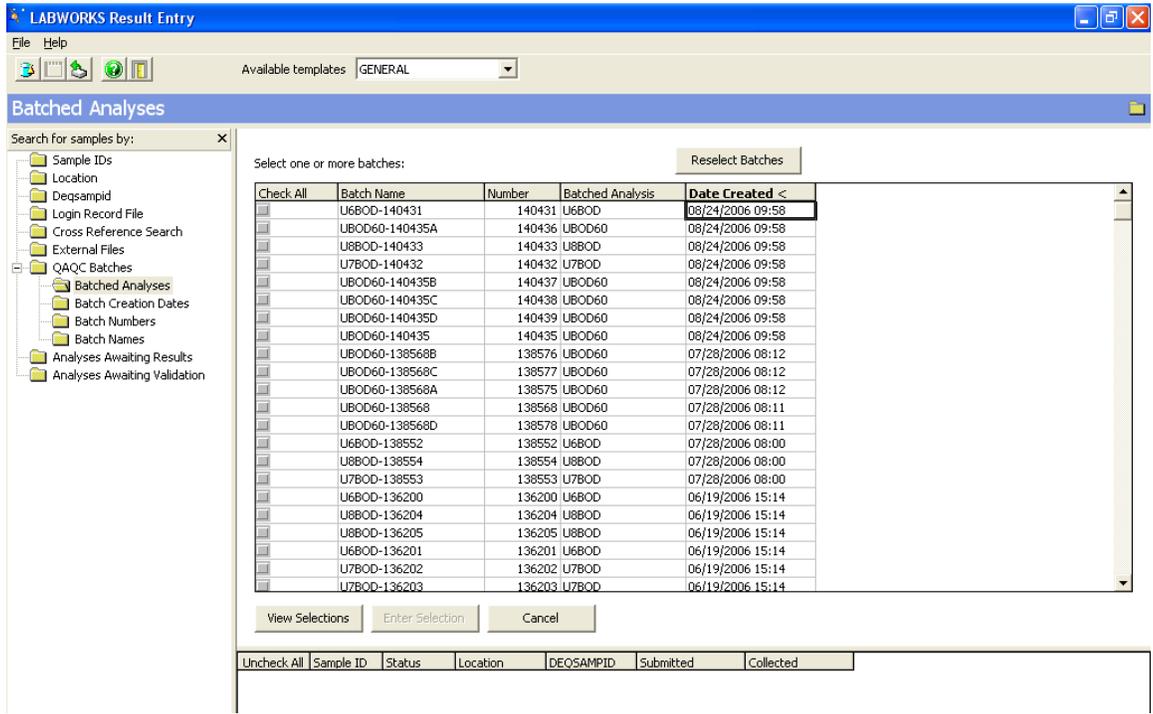


Figure 25

A3.7 Select the batch and cloned batches (only UBOD60 batch) that contain the samples ready for data input. Once the batches have been selected, click on **View Selections**. They will appear at the bottom of the screen. Choose **Enter Selection**. See Figure 26.

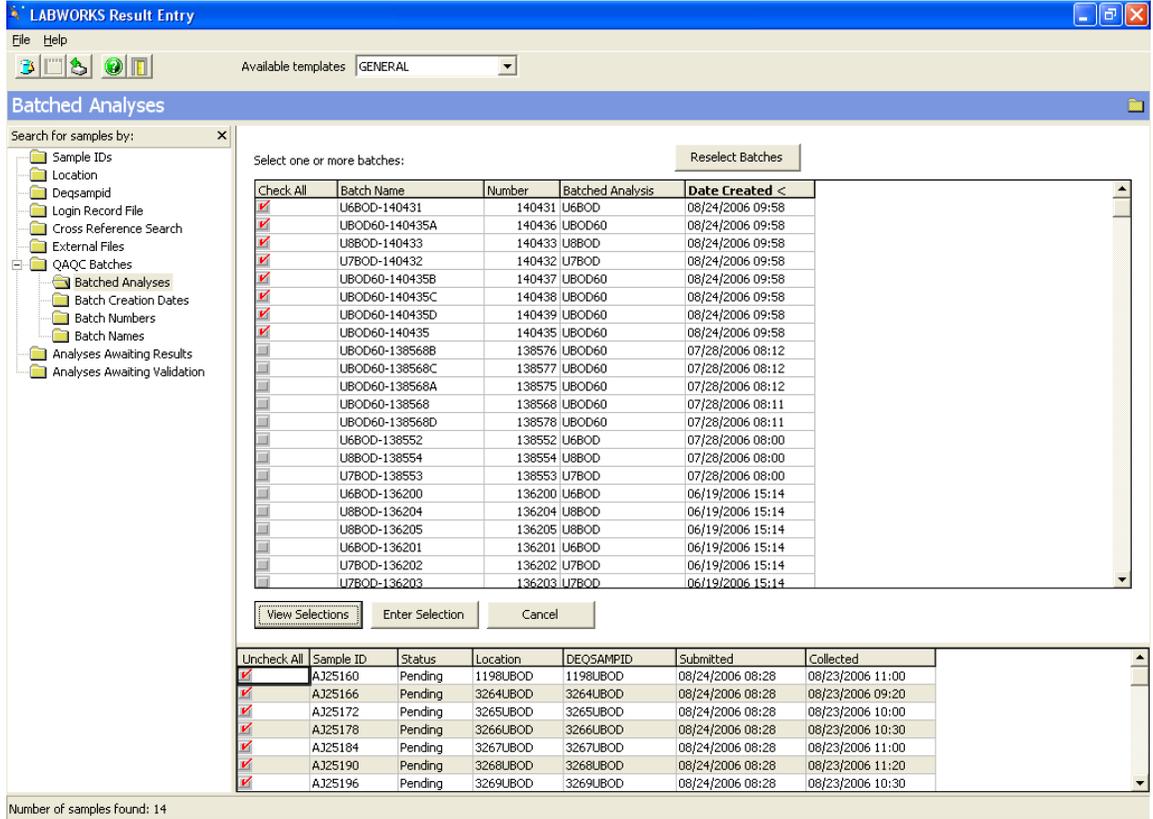


Figure 20

A3.8 The **Result Entry** screen will be displayed. The samples in the batch are listed in the rows the analyses selected are listed in the columns. Enter the results. Enter the calculated UBOD result from the final spreadsheet in the correct survey's folder found in the Q drive. Use the correct start and end dates/times. **The start date/time for every reading is the initial set-up date and time. The end date/time must correspond to the date/time that particular reading was taken.** See Figures 27, 28 and 29.

Result Entry:

Enter key action:
 None Right Down

Print Save Cancel

User Info	Anl Code	U6BOD	U7BOD	U8BOD	UBOD60	B_U1BOD	B_U2BOD	B_U
Meth Ref	5210B	5210B	5210B	5210B	5210B	5210B	5210B	5210B
Units	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Def MDL	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
AJ25160	result	9.5						
	Qualify							
	StartDate	08/24/2006						
	StartTime	13:00						
	EndDate	09/06/2006						
	EndTime	14:45						
	Analyst	CLP						
AJ25166	result	15.1						
	Qualify							
	StartDate	08/24/2006						
	StartTime	13:00						
	EndDate	09/06/2006						
	EndTime	14:45						
	Analyst	CLP						

Figure 27

	A	B	C	D	E	F	G
1							
2	Meter # 03A1314						Alabama Ba
3							
4			Reading 7	UBOD7	Reading 8	UBOD8	Reading 9
5			Day#: 20		Day#: 29		Day#: 40
6			Date: 9/14/6		Date: 9/22/6		Date: 10/3/6
7	Sample Number		Time: 0900		Time:		Time:
8	Site Number		Analyst: clp		Analyst:		Analyst:
9	BLANK #1			0.34		8.1	
10	12.5% Nutrients		7.76	0.3		8.1	
11	BLANK #2			0.35		8.17	
12	12.5% Nutrients		7.82	0.4		8.2	
13	# 1 - GGA			224.8		28.1	
14	12.5% Nutrients		3.67	225		28.1	
15	# 2 - GGA			219.3		30.6	
16	12.5% Nutrients		3.83	219		30.6	
17	AJ25160		7.1	10.83		18.01	
18	1198		7.18	10.8		18	
19	AJ25166		4.47	18.62		26.99	
20	3264		8.37	18.6		27	
21	AJ25172		7.18	8.32		15.52	
22	3265		7.2	8.3		15.5	

Figure 28

Result Entry:

Enter key action:
 None Right Down

Print Save Cancel

User Info	Anl Code	U6BOD	U7BOD	U8BOD	UBOD60	B_U1BOD	B_U2BOD	B_U3BOD	B_U4BOD	B_U5BOD	B_U6BOD	B_U7BOD
Meth Ref	5210B	5210B	5210B	5210B	5210B	5210B	5210B	5210B	5210B	5210B	5210B	5210B
Units	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Def MDL	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
AJ25160	result	9.5	10.8									
	Qualify											
	StartDate	08/24/2006	08/24/2006									
	StartTime	13:00	13:00									
	EndDate	09/06/2006	09/14/2006									
	EndTime	14:45	10:00									
	Analyst	CLP	CLP									
AJ25166	result	15.1	18.6									
	Qualify											
	StartDate	08/24/2006	08/24/2006									
	StartTime	13:00	13:00									
	EndDate	09/06/2006	09/14/2006									
	EndTime	14:45	10:00									
	Analyst	CLP	CLP									
AJ25172	result	7.0	8.3									
	Qualify											
	StartDate	08/24/2006	08/24/2006									
	StartTime	13:00	13:00									
	EndDate	09/06/2006	09/14/2006									
	EndTime	14:45	10:00									
	Analyst	CLP	CLP									
AJ25178	result	10.5	12.0									
	Qualify											
	StartDate	08/24/2006	08/24/2006									
	StartTime	13:00	13:00									
	EndDate	09/06/2006	09/14/2006									
	EndTime	14:45	10:00									
	Analyst	CLP	CLP									
AJ25184	result	12.8	14.4									
	Qualify											
	StartDate	08/24/2006	08/24/2006									

Left click result cell to enter data, headers to display user, test, or sample info Right click for context menu

Figure 29

- A3.9 **Sample comments** are used to report any violations of collection or storage, or failed QC results in the batch.
- A3.10 When all data have been entered, save the file to the LIMS system. For the Day 60 reading, print out the results after all data entry. The LABWORKS Result Entry screen will show after the samples are saved. If you want to view the samples again click on the tab that says **Enter results for selected samples**. See Figure 30.

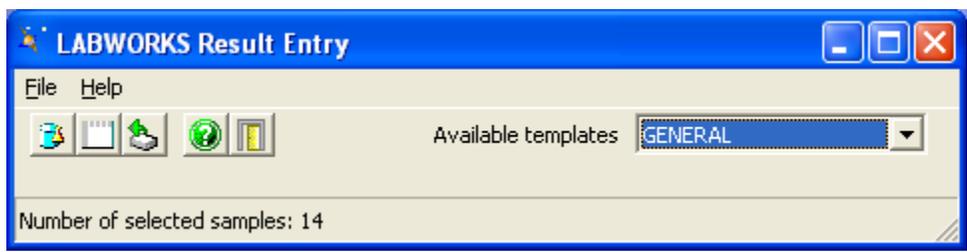


Figure 30

Appendix B Procedure for Calibration of the YSI meter

- B. 1 If the meter was turned off, turn on YSI meter approximately 30 minutes before using.
- B. 2 Aerate at least 3 liters of reagent water for 1 – 2 minutes. Aerated dilution water (set up day) can be used.
- B.3 When ready to calibrate the meter, fill three 300 ml BOD bottles with aerated reagent or dilution water.
- B.4 Place the probe into one of the bottles and turn on stirrer.
- B.5 Determine the dissolved oxygen of the other two bottles by Winkler Titration.
- B.6 Winkler Titration Procedure
 - 6.1 Add 2 ml manganous sulfate solution to each bottle (use a 3.5 ml plastic dropper) holding the pipette tip **above** the liquid surface when adding this reagent.
 - 6.2 Add 2 ml of alkali-iodide azide solution to each bottle (use a 3.5 ml plastic dropper) holding the pipette tip **below** the liquid surface when adding this reagent.
 - 6.3 Stopper carefully to exclude air bubbles.
 - 6.4 Mix by inverting the bottle 10-15 times.
 - 6.5 When precipitate has settled to approximately half the bottle volume, mix by inverting the bottle a second time and allow the precipitate to settle once again to half the bottle volume. Then add 2 ml of 75 % v/v sulfuric acid.
 - 6.6 Re-stopper and mix by inverting several times until precipitate dissolves completely. (A clear, golden solution)
 - 6.7 Measure 203 ml of the solution (using the special plastic volumetric flask) and transfer it to a 400 ml beaker with a stir bar in it.
 - 6.8 Titrate this solution with 0.025N sodium thiosulfate solution while stirring to a pale straw color.
 - 6.9 Add 5 drops of starch solution and continue titration to the first disappearance of blue color that does not return.
- B.7 After titration of both bottles, record the values titrated of both bottles into the logbook and average the values. The titrated values must be within the range of 0.2 mg/L. The average is the set point for the DO meter's calibration. Record once on each page in the calibration logbook the reagents' Standard ID number of each standard used for Winkler titration: Manganous Sulfate, Alkali-Iodide Azide, 75% Sulfuric Acid, Sodium Thiosulfate, and Starch Indicator. If a different solution is used, record on the page at the time of use.

B.8 Adjust the YSI meter to the value determined by the Winkler titration (mls of titrant equals mg/L DO). See Table 1: Oxygen Solubility and Calibration Table. NOTE: Be sure to check that the D.O. determined in the calibration does not exceed the value given in the Oxygen Solubility Chart for the temperature of the water. For example, if the water is at temperature 21.0 degrees C, the Dissolved Oxygen must be at or below 8.92. If the DO of the water exceeds the maximum for that temperature, the water is supersaturated and the D.O. must be reduced before using for analysis. Adding unaerated water is the easiest way to reduce D.O.

B.9 Accuracy of the temperature measurement of the DO meter and each probe in use must be verified yearly by immersing the probe in water at ~ 20 °C along with an NIST traceable thermometer and comparing readings. If any correction to the temperature measured by the probe is necessary, it must be noted and taken into account during calibration and analysis.

B.10 Calibrate the DO meter.

10.1 Press **Calibrate** button on the main menu.

Store	Review	Send	Calibrate
-------	--------	------	-----------

10.2 Press DO cal: The % number (top left number) starts flashing.

AutoCal	DO Cal	Setup	Diagnosis
---------	--------	-------	-----------

10.3 Press the “Next” button. The mg/l number (top right number) begins flashing.

Up	Down	Digit	Next
----	------	-------	------

10.4 Press the “Up” or “Down” buttons to get to the desired D.O. determined by the Winkler titration.

Up	Down	Digit	Next
----	------	-------	------

10.5 Once the DO is correct, press the Enter button to save the calibration value. Then press the Mode button once to go back to the main screen and begin reading DO.

Appendix C Calibration of Accumet AP61 pH meter

C 1.0 pH Standardization:

- 1.1 Press the “On/Off” key.
- 1.2 Press “setup” twice, then press “enter” to clear prior standardization.
- 1.3 Immerse electrode in buffer, press “std” to access standardize mode.
- 1.4 Press std. again to standardize. When standardization is complete, the buffer value is displayed.
- 1.5 Repeat with additional buffers for multi-point standardization.
- 1.6 After the last buffer is standardized, the efficiency of the electrode is shown. The acceptable electrode performance range is 90 -102, which is displayed as efficiency before returning to the measure mode.

C 2.0 pH Measuring – Auto On

- 2.1 Rinse electrode with reagent water. Blot excess.
- 2.2 Press mode until “pH” is displayed.
- 2.3 Press auto until “Auto” is displayed.
- 2.4 Immerse electrode in sample or LCS while stirring.
- 2.5 When “Stable” appears, the pH reading is locked in.
- 2.6 The measurement is now complete. Rinse electrode with reagent water. Blot excess.
- 2.7 Repeat steps 2.1 and 2.6 above for the pH measurement of additional samples.

C3.0 pH Monitoring – Auto Off

- 3.1 Press mode until “pH” is displayed.
- 3.2 Press auto until “Auto” is no longer displayed.
- 3.3 Immerse electrode in sample. Meter continuously monitors pH and displays reading.

APPENDIX D

Troubleshooting

- D.1 After initial calibration, if BOD meter and probe do not hold the calibration from one day to the next within acceptable limits, check the results by titrating at least one bottle using the Winkler titration to verify that the titration results agree with the probe reading. Significant adjustments in the calibration needed from day to day may indicate that the probe (or meter) needs repair.
- D.2 If the probe readings on the meter are not stable and tend to jump around, check for bubbles in the membrane by tilting the probe back and forth. If there is a bubble under the membrane, the membrane cap needs to be changed.
- D.3 If blanks give a UBOD of more than 0.2 ppm in 5 days or 1.0 ppm in 60 days, this may indicate that the dilution water jugs and BOD bottles were not properly cleaned or there is a problem with the dilution water source. Rinse the plastic jug with a small amount of 3 N HCl. Rinse the jug with reagent water before filling the jug. Also, check the BOD bottles to see how clean they are by holding the bottle up to the light. If there is a film present on the bottom or the sides of the bottle, then it needs to be cleaned. To clean the bottles, rinse with small amount of 3N HCl and large amounts of hot water with a final reagent water rinse. If film is still present, scrub with a brush until it goes away and then put through the wash cycle. Consult with the Scientist Manager and document the failure. Manual calculations must be done to offset the contamination.
- D.4 If the blank DO reading is higher than the previous DO reading, the meter calibration may need to be checked. If the increase in the Blank DO is greater than 0.05, the calibration needs to be rechecked. No action needs to be taken if the blank reads up to 0.04 higher than previous DO. To check the meter calibration, do an air calibration (see YSI instrument manual) and compare the air calibration DO with the Winkler titration DO to see if they are the same. If they are relatively close to the same, then there are no problems with the reagents or the calibration. If they are not the same repeat the Winkler Titration. If the number still did not change, make a fresh 0.1N Sodium thiosulfate (from a different lot, if available) and a fresh 0.025 N sodium thiosulfate, and repeat the Winkler titration with the new reagents. If after checking the calibration and the reagents no improvement is seen, contact the supervisor or manager before proceeding.
- D.5 If samples are supersaturated with oxygen, make sure they are warmed up to ~ 20°C and then aerate the sample to lower the dissolved oxygen, especially before setting up dilutions of the samples.

APPENDIX E - Procedure for washing glassware

- E.1 2 L BOD bottles – 2 L glass bottles with ground-glass stoppers. Before use in an analysis, bottles must be washed according to the following instructions:
1. Remove any labels with acetone.
 2. Push the yellow button to open the door.
 3. Place the bottles in Miele glassware washer.
 4. Push the yellow button to close the door.
 5. Be sure the 8 “B” program is selected. This is the UBOD bottle wash program.
 6. Depress the green Start button located at the bottom right corner of the control panel. Indicator lights will show what stage the wash is in.
 7. The entire cycle takes about one hour.
- E.2 When bottles are finished, take them out of the washer. Stopper and store each bottle in the cabinet.

APPENDIX F

Dechlorination Procedure for Chlorine

- F.1 Measure 100 ml of sample into a 150 ml beaker.
- F.2 Add 1 ml of 2% H₂SO₄. Swirl.
- F.3 Add 1 ml of potassium iodide (KI) solution. Swirl.
- F.4 Add 3 drops of starch indicator.
- F.5 Titrate with 0.0125N Na₂SO₃ (sodium sulfite) solution until the blue color disappears. The volume of Na₂SO₃ used in the titration is the volume of Na₂SO₃ to be added to the original sample per 100 ml.
- F.6 Add sufficient sodium sulfite to dechlorinate the entire sample. Stir for 10 min. and check for chlorine again. If chlorine is still present, add additional sodium sulfite, stir and check until no chlorine is present. Record the amount of sodium sulfite used to dechlorinate.