



STANDARD METHODS
FOR THE
EXAMINATION OF WATER AND WASTEWATER
JOINT EDITORIAL BOARD
MEMORANDUM

To: *Standard Methods* Users
Biochemical Oxygen Demand (BOD)

From: Rodger Baird
Joint Editorial Board (JEB)

Re: BOD Dilution Series

Date: May 13, 2009

This letter is in response to questions about the changes in the 20th and 21st Editions of *Standard Methods* regarding the recommended dilution series in the 5-day BOD test, Method 5210B. As described below, it was never the intention of the Standard Methods Joint Task Group for 5210B to make it mandatory that more than one dilution per sample meet the DO criteria.

The 19th and earlier editions recommended making several dilutions in order to insure that at least one met the DO uptake criteria of at least 2.0 mg/L depletion and a residual of 1.0 mg/L. The 20th Edition was edited to recommend up to five dilutions of unknown matrices be made in order to get two dilutions that met the DO criteria. The 21st edition was edited again to recommend at least three dilutions be made that meet the DO criteria. The language is as follows for the two later editions:

20th Edition, 5210B.4.f: “Make several dilutions of sample that will result in a residual DO of at least 1.0 mg/L and a DO uptake of at least 2.0 mg/L after a 5-d incubation. Five dilutions are recommended unless experience with a particular sample shows that use of a smaller number of dilutions produces at least two bottles giving acceptable minimum DO depletion and residual limits.”

5210B.5.: “If more than one sample dilution meets the criteria of a residual DO of at least 1 mg/L and a DO depletion of at least 2 mg/L,.... average results in the acceptable range.”

21st Edition, 5210B.5.c: “Using the dilution water prepared in ¶ 5a, make at least three dilutions of prepared sample estimated to produce a residual DO of at least 1.0 mg/L and a DO uptake of at least 2.0 mg/L after a 5-d incubation. Five dilutions are recommended if experience with a particular sample does not produce at least three bottles having acceptable minimum DO depletion and residual limits (¶ 6a).”

After JEB discussion with the Part Coordinator and Joint Task Group Chair for Section 5210, it has been determined that the recommendations made regarding the number of dilutions meeting the DO criteria in the 20th and 21st Editions were not intended to represent a pass-fail criterion for the BOD test results. It is important to understand that the sole purpose of sample dilution in the BOD test is to insure that a valid sample aliquot is tested in this 5-day test, because if the DO window is missed,

the sample can not be re-run with validity. The presence of additional dilutions that meet the DO criteria have an adjunct benefit in some cases if sample toxicity is revealed, but this is a rare occurrence. There is no statistical advantage to “averaging” results from several dilutions because they are not true replicates in either a statistical or analytical sense. They are averaged if they all meet test criteria only because it cannot be determined that one result is “better” than another.

As additional support that the recommended changes were not intended to be prescriptive in a pass-fail sense, the calculation section of the 20th Edition quoted above says “*If* more than one sample dilution meets the criteria (for DO)....average results ...”. And in the 21st Edition, the language cited above says “...make at least three dilutions...*estimated* to produce a residual DO of.....”. [emphasis added]. Clearly, the analyst is being instructed to estimate that the dilution series will fall into the necessary DO range, an expectation that can only be found out after five days. In other words, the instructions are a “due-diligence” guide for the analyst. Because of holding-time considerations, samples cannot be re-tested after the 5-d results are known, and it was never the intention of the Standard Methods Committee or Joint Task Group to arbitrarily create either a procedure change that would cause test failure, or a regulatory sticking point for laboratories. The guidance represented in these editorial changes of the 20th and 21st Editions of 5210B were, rather, made to help analysts (particularly, inexperienced analysts) achieve a valid BOD test result.

Very Truly Yours,

Rodger Baird
Joint Editorial Board
Standard Methods for the Examination of Water and Wastewater