



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

TO: *Standard Methods* Users
Biochemical Oxygen Demand

FROM: Andrew Eaton
Andrew Eaton
Joint Editorial Board

RE: BOD Sample Dilution Procedures

DATE: June 30, 2015

This letter is in response to multiple questions about dilution preparation procedures using volumetric containers or BOD bottles in the *Standard Methods* 5210B – 2011 method. The method states in Section 5210B.5.c.1: “Dilutions prepared in volumetric containers – Using a wide-tipped pipet, add desired amount of prepared sample to individual volumetric cylinders or flasks. Mix the sample well immediately before pipetting to avoid loss of solids by settling. For dilutions greater than 1:100 make a primary dilution before making final dilution in the bottle.” Section 5210B.5.c.2 states: “Dilutions prepared directly in BOD bottles – Using a wide-tipped volumetric pipet, add the desired sample volume to individual BOD bottles. Fill each BOD bottle approximately two-thirds full with dilution water. Add appropriate amounts of seed suspension and nitrification inhibitor to the individual BOD bottles. When a bottle contains more than 67% of the sample after dilution, nutrients may be limited in the diluted sample and subsequently reduce biological activity. In such samples, add the nutrient, mineral and buffer solutions (5210B.3a-e) directly to diluted sample at a rate of 1 mL/L or use commercially prepared solutions designed to dose the appropriate bottle size.”

Based on the above statements, the requirements are different between dilutions prepared in volumetric containers and BOD bottles. For dilutions prepared in volumetric containers, the method states that the sample is mixed well immediately before pipetting to avoid loss of solids by settling and that dilutions greater than 1:100 require a primary dilution. For dilutions prepared in BOD bottles, dilutions containing greater than 67% sample volume require the addition of nutrient, mineral and buffer solutions at a rate of 1 mL/L due to the potential of limited nutrients in the sample.

Standard Methods would like to clarify that the following apply regardless of whether the sample dilution is prepared in a volumetric container or a bottle:

1. Mix the sample well immediately before pipetting to avoid loss of solids by settling.
2. For dilutions greater than 1:100 make a primary dilution before making final dilution in container/bottle.
3. When a container/bottle contains more than 67% of the sample after dilution, nutrients may be limited in the diluted sample and subsequently reduce biological activity. In such samples, add the nutrient, mineral, and buffer solutions directly to diluted sample as a rate of 1 mL/ L or use commercially prepared solutions designed to dose the appropriate container/bottle size.

When we publish the next revision to the BOD method this will be clarified in the text. In the interim, this guidance will serve as a statement of the *Standard Methods* policy on this issue.