BOD_5

Preparing the dilution water (supply water) for the LCK555 cuvette test

APP-PHM-0010 application

EN

General
In the case of lower findings and / or variance with the conventional working method, we recommend the use of fresh supply water for the creation of the dilution water. These instructions are an addition to the working procedure of the Hach Lange LCK555 cuvette test and do not replace it.

CHANGES:

- ***Extended*** *supply water service life and aeration time (already several days prior to the actual test run)*
- *Inoculation* ***after*** *the aeration*

1. **Extended** supply water **aeration**

In order to completely remove chlorine, its by-products or other interfering matter, the supply water used for the creation of the dilution water must be aerated for at least **2 days.** This prevents damage (inhibition) from any bacteria introduced during the process.
Larger quantities can be processed here (up to 4 liters were tested). The aerated supply water can be kept in a darkened storage vessel for up to 4 weeks.

2. Completing the dilution water – **inoculation after aeration**

On the day of the BOD_5 process:

a. Preparing the aerated supply water

- Put **500 ml** of aerated supply water (from the storage vessel) in a plastic container (LZC901)
- Aerate for **1 hour** (this hour is not applicable if the aerated supply water is used directly after the 2-day aeration process)
- Add **0.3 ml** of the trace element (bottle D, LCK555) using a pipette and gently swirl to mix
- Leave for **1 hour** without aeration to prevent oxygen saturation. (During this time, create inoculation solution (point 2 b) and standard (point 3); see below.)

b. Creating the inoculation solution (BioKit LZC555)

If Lange BioKit LZC555 is used as inoculation material, create the **inoculation solution** as follows while the prepared supply water is standing for an hour:

- Put **1 level measuring spoon** (blue) of inoculation material (A) in a vial
- Add **10 ml** of buffer using a pipette (B)
- Seal the vial and shake vigorously for **1 min** (oxygen enrichment)
- Leave for **1 hour** (encourages resuspension of the bacteria)

c. Inoculation

Despite the current working procedure, the **inoculation** is only carried out **after the aeration** and after the prepared supply water has stood for one hour.

- Put 0.5 ml of the excess settled inoculation solution or a corresponding amount of another inoculation solution **with no particles** in the prepared supply water and gently swirl to mix

3. Preparation of the **standard** (LCA 555) and **sample**

This preparation process can be carried out while the prepared supply water and the inoculation solution is standing for an hour.

a. Standard solution (target value 205 +/- 45 mg/l)

- Put 5.0 ml of demineralized water in a cuvette with lyophilisate (LCA555) using a pipette
- Seal the cuvette and shake vigorously until the lyophilisate has dissolved
- After **3 minutes, put 2.2 ml of this solution** in a 50 ml volumetric flask using a pipette, fill with demineralized water and shake well

b. Sample: Prepare the sample according to the dilution table (working procedure LCK 555)

4. Prepare **test series**

Important: *When filling, ensure that there is no air in the cuvette, i.e. the dilution water should form an upward curving meniscus at the cuvette opening*

- **Dilution water**: Fill cuvettes (LCK 555) with the dilution water using a transfer pipette, ensuring there are no air bubbles, and carefully seal with the DosiCapZip
- **Standard**: Put 0.5 ml of standard solution in the cuvettes (LCK 555), then fill with dilution water using a transfer pipette, ensuring there are no air bubbles, and seal with the DosiCapZip
- **Sample**: Put 0.5 ml of the prepared sample solution in the cuvettes (LCK 555), then fill with dilution water using a transfer pipette, ensuring there are no air bubbles, and seal with the DosiCapZip

Incubate the cuvettes in a temperature chamber at 20°C **for 5 days**.

5. **Analysis** after 5 days: see working procedure LCK 555

6. **Definition of terms**

- **Supply water**
  Fresh water, taken from the supply
- **Aerated supply water**
  Supply water that has been aerated for at least two days
- **Prepared supply water**
  Aerated supply water with trace elements removed
- **Dilution water**
  Aerated supply water with trace elements removed, which has been inoculated with bacteria

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