Measuring Ammonia using HACH® Ammonia Nitrogen Test Kits for Freshwater and Saltwater Samples

1. SCOPE AND APPLICATION

1.1. These methods are adapted from the HACH® Ammonia Nitrogen Test Kit instruction manuals. These methods are used to determine ammonia in both freshwater and saltwater aqueous samples.

1.2. Use of this SOP constitutes acknowledgement that the HACH® Ammonia Nitrogen Test Kit NI-8 (0-3mg/L) and HACH® Ammonia Nitrogen Test Kit NI-SA (0-2.5mg/L) user's manuals have been read and understood by the operator.

2. SUMMARY OF METHOD

2.1. A color disc is placed in the color comparator box.

2.2. Sample is placed in two plastic viewing tubes.

2.3. For freshwater samples: Test kit NI-8 (0-3mg/L)

2.3.1. Standards of known concentrations are tested; 1 PPM and 3 PPM, as well as a "0" blank using DI water. One tube is placed in the left opening of the color comparator box; Nessler reagent is added to the second tube and swirled to mix. After 1 to 5 minutes, the second tube is placed in the right opening of the color comparator box. The comparator box is held in front of a light source and the color disc is turned until there is a color match. Results are read and recorded.

2.3.2. Interferences include:

2.3.2.1. Contamination when tubes are not thoroughly rinsed after each use

2.3.2.2. A wet disc. If disc is wet, disassemble and use a soft cloth to dry all parts. Reassemble when fully dry.

2.3.2.3. Temperatures deviating from 20 ºC. Warmer temperatures cause high results while lower temperatures cause low results.

2.3.2.4. More than 6 gpg (grain per gallon / ~102 mg $CaCO_3$/L) hardness causes a white precipitate to develop. To remove the interference, add 1 drop Rochelle salt reagent to the tube.

2.4. For saltwater samples: Test Kit NI-SA (0-2.5mg/L)

2.4.1. Standards of known concentrations are tested; 1 PPM and 2 PPM, as well as a "0" blank using artificial salt water. One tube is placed in the left opening of the color comparator box. One ammonia salicylate powder pillow is added to the other tube and shaken. After 3 minutes, one ammonia cyanurate powder pillow is added to the same tube and shaken. After 15

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minutes, tube is placed in the right opening of the color comparator box. The comparator box is held in front of a light source and the color disc is turned until there is a color match. Results are read and recorded.

2.4.2. Interferences specific to saltwater samples include:

2.4.2.1. Measuring brackish water, resulting in readings higher than actual value. With this kit, error should be <10%.

2.4.2.2. Measuring low salinity or freshwater, resulting in readings higher than actual value. With this kit, error can be as high as 16%.

2.5. If results are unusually high or inconsistent, a replicate is necessary.

3. EQUIPMENT AND SUPPLIES

3.1. Disposable pipette (10mL) with manual pipettor

3.2. Laboratory timer (count-down or count-up), with alarm

3.3. For freshwater samples: HACH® Ammonia Nitrogen Test Kit NI-8 (0-3mg/L) containing:

3.3.1. Two 18mm plastic viewing tubes

3.3.2. One color disc

3.3.3. One color comparator box

3.3.4. One bottle of Nessler reagent

3.4. For saltwater samples: HACH® Ammonia Nitrogen Test Kit NI-SA (0-2.5mg/L) containing:

3.4.1. Two 18mm plastic viewing tubes

3.4.2. One color disc

3.4.3. One color comparator box

3.4.4. Ammonia Salicylate Reagent Powder pillows

3.4.5. Ammonia Cyanurate Reagent Powder pillows

3.4.6. Two stopper caps for tubes

4. REAGENTS AND STANDARDS

4.1. Deionized (DI) water, for dilution of higher-level samples and for thoroughly rinsing viewing tubes

4.2. Nessler reagent, for freshwater samples.

4.3. Ammonia Salicylate Reagent, for saltwater samples

4.4. Ammonia Cyanurate Reagent Powder pillows, for saltwater samples.

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4.5. Ammonia working standards 1 PPM and 3 PPM for freshwater samples

4.6. Ammonia working standards 1 PPM and 2 PPM for saltwater samples.

5. METHOD

5.1. Standards are read before sample water to assure quality methods. For freshwater samples, first read DI water as a "0" blank. Ammonia concentration should read 0 for DI water. Follow **STEP 5.7** using freshwater ammonia working standards 1 PPM and 3 PPM instead of sample water. Read result according to **STEP 5.9** and record result in the ammonia logbook. Standards should read 1 PPM and 3 PPM respectively, if they do not then new standards should be made according to **STEP 5.2 and 5.3**. For saltwater samples, read artificial saltwater as a "0" blank. Follow **STEP 5.8** using saltwater ammonia working standards 1PPM and 2 PPM instead of sample water. Read the result according to **STEP 5.9** and record result in the ammonia logbook. Standards should read 1 PPM and 2 PPM respectively, if they do not then new standards should be made according to **STEP 5.2 and 5.3.**

5.2. Freshwater working standards:

5.2.1. For the 1PPM standard, measure out 12 ml per 100 ml of DI water.

5.2.2. For the 3PPM standard, measure out 36 ml per 100 ml of DI water.

5.3. Saltwater working standards:

5.3.1. For the 1 PPM standard, measure out 12 ml per 100 ml of ASW.

5.3.2. For the 2 PPM standard, measure out 24 ml per 100 ml of ASW.

5.4. Keep samples at room temperature

5.4.1. Warmer temperatures cause high results while colder temperatures cause low results. (See table in NI-SA kit instruction manual).

5.5. Use the disposable pipette with the manual pipettor to add 5mL of sample water into each plastic viewing tube.

5.6. Place one plastic viewing tube into the left opening of the color comparator box, farthest from the color disc.

5.7. For freshwater samples (refer to Step 5.6 for saltwater samples):

5.7.1. Add three drops of Nessler reagent to the tube that is not in the comparator box. Do not shake the Nessler reagent bottle.

5.7.2. Gently swirl the tube containing Nessler reagent until a yellow color develops.

5.7.3. Wait at least one minute, and no more than five minutes, to read the results. Refer to **STEP 5.9** on how to read results.

5.8. For saltwater samples:

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5.8.1. Add one ammonia salicylate powder pillow to the tube that is not in the comparator box.

5.8.2. Cap tube and shake until all powder is dissolved.

5.8.3. Wait three minutes.

5.8.4. To the same tube, add one ammonia cyanurate powder pillow.

5.8.5. Cap tube and shake until all powder is dissolved.

5.8.6. Wait 15 minutes before reading results.

5.8.6.1. Refer to **STEP 5.9** on how to read results.

5.8.6.2. If used for brackish or freshwater, the reading will be slightly higher than the actual value.

5.9. Reading results for freshwater and saltwater samples:

5.9.1. Put the second tube (with Nessler reagent or powder pillows) into the open slot of the color comparator box, closest to the color disc.

5.9.2. Hold color comparator box, with both tubes inside, in front of a light source. Compare the front two color viewing windows.

5.9.2.1. These windows are in-line vertically with the viewing tubes and next to each other. They are located on the left side midway from the top and bottom of the color comparator box.

5.9.3. While looking at the color viewing windows, turn the color disc until the colors in the front windows match. Record the result at the point in which the colors match.

5.9.3.1. If the color match in between two segments, use the value that is in the middle of the two segments.

5.9.3.2. The result can be found in a single window just below and slightly to the right of the color viewing windows mentioned in **STEPS 5.9.2.** and **5.9.2.1**.

5.10. Record the results in the Ammonia logbook and the appropriate bench sheets.

5.10.1. If an out-of-range result is found (>3 mg/L freshwater kit; >2.5 mg/L saltwater kit), dilute a freshwater sample with DI water and a marine sample with ASW of similar salinity. Perform steps for either freshwater (**STEP 5.7**) or saltwater (**STEP 5.8**) as appropriate on diluted sample. Multiply the result by the dilution factor.

5.10.2. Readings less than 0.2 mg/L are recorded as <0.2 for both kits. Readings >0.2 mg/L are recorded as displayed on the results window.

5.10.3. If saltwater results are unusually high or inconsistent, a replicate is necessary. For the saltwater HACH® Ammonia Nitrogen Test Kit NI-SA, a

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lower result on the second test indicates contamination of the first test. Troubleshoot and re-run the sample.

5.11. Rinse viewing tubes thoroughly with DI water then repeat **STEP 5.7 or 5.8** for the replicate sample, if needed.

5.12. When finished with the kits, rinse the viewing tubes thoroughly with DI water and allow to dry before placing in kit. If Nessler reagent was used, ensure the lid is secure. Return dry viewing tubes, color comparator boxes, Nessler reagent and any unused pillow packets to appropriate HACH® ammonia kit. Clean up the work area.

6. DATA ARCHIVAL

6.1. Use a black, waterproof pen for all bench sheets and logbooks.

6.2. To correct raw data entries, place a single line through the incorrect or illegible entry, write the corrected entry near the error, date and initial the correction. Do not overwrite entries.

6.2.1. The corrected entry is written as near as possible to the error or in the comment box on the appropriate bench sheet.

6.2.2. If the correction requires an explanation, write a comment and reference the error with a letter. Initial and date the explanation. Do not attempt to put all information (correction and comments) into a small space.

6.2.3. Use sequential alphabetical annotation to identify errors and corresponding comments (use A for first error and use A for corresponding explanatory comment; use B for second error, etc.).

6.3. Record information in “TOX_AMMONIA” logbook in the Laboratory Information Management System (LIMS) and associated *Bioassay Bench Sheets*.

7. QUALITY CONTROL

7.1. Refer to QA manual for corrective action and handling out-of-control data.

7.2. If a problem occurs during the sample processing or testing, the following steps are performed: 1) Lab supervisor is notified. 2) Client is notified (client makes decision for additional testing). 3) Section administrator is notified. 4) A non-conformance report (NCR) is submitted describing the problem and the corrective action.

7.2.1. To create NCR: open LIMS and go to Laboratory/Administrative/Non-Conformance Report. Refer to https://floridadep.gov/dear/quality-assurance/content/dep-sops for sample collection and

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preservation, and the Laboratory Quality Manual for corrective action and handling out-of-control data.

8. SAFETY/HAZARDOUS WASTE MANAGEMENT

8.1. Proper laboratory protection, including lab hood or ventilation system, lab coat, gloves and safety glasses, is required when working with chemicals and unprocessed samples.

8.2. Refer to the Laboratory Quality Manual for sample disposal.

8.3. Refer to the Laboratory Health and Safety Manual and Hazardous Waste Management.

9. REFERENCES

9.1. HACH® Ammonia Nitrogen Test Kit NI-8 (0-3mg/L mid-range) Instruction Manual.

9.2. HACH® Ammonia Nitrogen Test Kit NI-SA (0-2.5mg/L) Instruction Manual

9.3. Laboratory Quality Manual, https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops

9.4. Laboratory Health and Safety Manual and Hazardous Waste Management health-safety-manual (floridadep.net)

9.5. DEP SOP MB-03.3, *Calibration and Use of Mechanical and Disposable Pipettes.*

APPENDIX OF CHANGES:

12/05/2016 SOP written using TA-6.22-2.17 *Measuring Total Residual Chlorine using the Hach® Pocket Colorimeter™ and Colorimeter™ II Test Kits* as a template. Also, formatted for 508 compliance.

10/06/2017 Updated links.

10/16/2018 Updated links.

10/02/2019 Changed 4.6 to correct standard (2 ppm not 3 ppm). Changed font to Calibri.

10/16/2020 Updated Data Archival and Quality Control sections, rearranged method format, and updated links.

9/27/2021 Updated Links and SOP reference, and changed font to match.

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