Measuring Total Ammonia using the Hach® Ammonia Nitrogen Pocket Colorimeter™ II Test Kit

1. SCOPE AND APPLICATION

1.1. This method is based on the Hach® Ammonia Nitrogen Pocket Colorimeter™ II Test Kits instruction manuals.

1.2. Use of this SOP constitutes acknowledgement that the Hach® Ammonia Nitrogen Pocket Colorimeter™ user's manuals have been read and understood by the operator.

1.3. This laboratory is not NELAC certified for this analysis. Note: Results of ammonia measurements performed on samples in the Bioassay laboratory are not reportable for compliance purposes. The results are used to determine potential causes of observed toxicity.

2. SUMMARY OF METHOD

2.1. Operation of the meter is checked. Standards are checked (blank, low, and medium). Sample is pipetted into sample cell and blank is prepared. Salicylate reagent is added to the sample and blank cells. Then the Timer is set for three minutes. Cyanurate reagent is added to the sample and blank cells. The Timer is set for fifteen minutes. The instrument is blanked. Sample cell is placed into meter and the "Read" button is pressed to get the value in mg/L of NH3.

2.2. Interferences:

2.2.1. Contamination when cells are not thoroughly rinsed with DI water after each use, resulting in a second reading lower than the first.

2.2.2. Sample contains an interfering substance such as iron or monochloramine (see manual for more substances).

2.2.3. Fingerprints or other smudges on the outside of the chamber.

2.2.4. Damage to the meter by water/liquid entering the cell holder.

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

3. EQUIPMENT AND SUPPLIES

3.1. Hach® Ammonia Nitrogen Pocket Colorimeter™ II Lot: A8051

3.2. 10 ml 25 mm x 60 mm sample cells with lids (two per sample and one blank)

3.3. 10 mL volumetric pipet with manual or automatic pipettor

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3.3.1. One per sample to be tested (volume of each new pipette lot is checked per SOP TA-06.32)

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

3.5. Computer with access to the Laboratory Information Management System (LIMS)

3.6. *Parameter Check* logbooks

3.7. *Bioassay Bench Sheets* logbooks (when applicable)

3.8. Kimwipe®

4. REAGENTS AND STANDARDS

4.1. Hach®-SpecV™ Secondary

4.1.1. The Hach® secondary standards come four to a box and include a blank, a low-range standard, a mid-range standard, and high-range standard. For our laboratory purposes, only the blank, low-range and mid-range are used.

4.2. Ammonia Salicylate Reagent Powder pillows (specifically made for a 10 mL volume of sample)

4.3. Ammonia Cyanurate Reagent Powder pillows (specifically made for a 10 mL volume of sample)

5. METHOD

5.1. Meter Calibration

5.1.1. Lab colorimeters are calibrated before their first use, and periodically throughout service. Colorimeters are also calibrated whenever the standard measurements begin to drift outside the acceptable range. (The acceptable value for standard measurements is ±10%.)

5.1.2. The colorimeter is turned on by pressing and releasing the [Power/Backlight Key] ( ). The display will turn on and show the value of the last reading. If the colorimeter has been calibrated, the [Calibration Adjusted Icon] ( ) will display on the right-side perimeter of the screen.

5.1.3. To enter calibration mode, press and hold the [Menu Key] ( ) until "USER CAL" is displayed and press the [Read/Enter Key] (✓). "S0" should appear on the display.

5.1.4. Place the Blank Standard in the cell holder, cap the colorimeter, and press the [Read/Enter Key] (✓). If not already at 00.00, using the [Zero/Scroll Key] ( ) manually input the standard value of the blank

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(00.00). Press the [Read/Enter Key] (✓) to cycle through the digits. Once the value is correctly entered, A0 should appear on the display.

5.1.5. Press the [Read/Enter Key] (✓) to measure the absorbance value of the blank.

5.1.6. Press the [Zero/Scroll Key] ( ) to move to the next calibration point. S1 should display on the screen.

5.1.7. Place “STD 1” in the cell holder and cap the colorimeter. Press the [Read/Enter Key] (✓) to cycle through the digits and use the [Zero/Scroll key] ( ) to manually enter the standard value of “STD 1” (Each standard value is written on the inside cap of the Standards box).Press the [Read/Enter Key] (✓), A1 should appear on the display after the standard value is entered.

5.1.8. Press the [Read/Enter Key] (✓) to measure the absorbance value of “STD 1”.

5.1.9. Press the [Zero/Scroll Key] ( ) and “Add” should appear on the display (S2 may appear if the meter has already been calibrated). Press the [Read/Enter Key] (✓) and “S2” should appear on the display.

5.1.10. Place “STD 2” in the cell holder and cap the colorimeter. Press the [Read/Enter Key] (✓) and use the [Zero/Scroll key] ( ) to manually enter the standard value of “STD 2”. “A2” should appear on the display after the standard value is entered.

5.1.11. Press the [Read/Enter Key] (✓) to measure the absorbance value of “STD 2”.

5.1.12. Once the absorbance value of “STD 2” is displayed, press the [Menu Key] ( ) twice quickly to return to measurement mode.

5.1.13. The display will flash the [Zero/Scroll Key] symbol ( ). Insert the Blank Standard into the cell holder and press the [Zero/Scroll Key] ( ).

5.1.14. The display will show 0.00 and the [Calibration Adjusted Icon] ( ) will appear on the right perimeter of the display.

5.1.15. Read Standards 1, 2, and 3 to check the calibration of the Colorimeter.

5.2. Remove Calibration

5.2.1. To delete a calibration, turn the colorimeter on, then press and hold the [Menu Key] ( ) until “USER CAL” appears.

5.2.2. Press the [Zero/Scroll Key] ( ) until “dFL” appears on the screen. This will return the Colorimeter to the factory default calibration setting.

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5.2.3. The display will flash the [Zero/Scroll Key] symbol ( ). Press the Zero button and the display will show 0.00 and the [Calibration Adjusted Icon] ( ) will no longer appear on the right perimeter of the display.

5.3. Calibration Verification

5.3.1. Press the power button on the Colorimeter™II. The display will turn on.

5.3.1.1. Ensure that the standards have not expired.

5.3.2. Remove the cap from the instrument. Wipe the Specv™ blank with a clean Kimwipe® and place into the cell holder. Ensure that the [Alignment Mark] ( ) on the blank standard is facing the keypad. Securely cover the cell with the instrument cover.

5.3.3. Press the [Zero/Scroll Key] ( ). The display will show "0.00".

5.3.4. Remove the instrument cap. Remove the Specv™ blank from the cell holder. Place the STD 1 cell into the cell holder after wiping it with a clean Kimwipe®, aligning the alignment mark with the keypad. Securely cover the cell with the instrument cover.

5.3.5. Press [Read/Enter Key] (✓). Record the displayed value in the LIMS/Laboratory/Technical/Laboratory Logbook/TOX_AMMONIA.

5.3.5.1. If the value is not within ±10 % of the standard value (The standard values are written on the inside lid of the standards box or on the certificate of analysis provided with the standards). Ensure that the standard cells are clean and repeat the procedure. If values are still out of range, refer to the instrument manual for trouble shooting instructions.

5.3.6. Repeat STEP 5.3.4. and STEP 5.3.5, with the Specv™ cell labeled STD 2.

5.4. Measuring Total Nitrogen, Ammonia

5.4.1. Use a 10 mL pipet to place 10 mL of DI water into an empty sample cell. This is the blank for all samples in the run.

5.4.2. Use a 10 mL pipet to place 10 mL of sample into an empty sample cell (use two cells per sample).

5.4.2.1. If measuring multiple water types/samples (facility sample, fish water, control water, etc.), use one pipet per sample (do not cross-contaminate samples).

5.4.3. Open one Ammonium Salicylate powder pillow per cell and add the contents to the sample cells and the blank cell, then secure the caps.

5.4.3.1. Two sample cells contain the sample and the sample duplicate. Additional samples can be run at the same time,

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using two cells per sample. Use appropriate number of powder pillow packets to fill each cell.

5.4.4. Shake to dissolve the powder then set a 3-minute timer.

5.4.5. After three minutes, open one Ammonium Cyanurate powder pillow per cell and add the contents to the sample cells and secure the caps.

5.4.6. Shake to dissolve the powder then set a 15-minute timer.

5.4.6.1. Check the sample chamber for bubbles that sometimes form on the sides of the chamber. These bubbles will cause a false high result. If bubbles are seen, gently tap the side of the chamber and swirl the contents of the chamber to dislodge the bubbles.

5.4.7. After 15 minutes, wipe the exterior of each cell with a Kimwipe® to remove any dirt, smudges, fingerprints, etc. which may reduce the amount of light passing through the cell, thereby giving an inaccurate reading.

5.4.8. Place the sample cell containing the blank sample in the cell holder. Place the instrument cap over the sample cell.

5.4.8.1. The [Alignment Mark] ( ) on the sample chamber should be facing the front of the instrument.

5.4.9. Press the [Zero/Scroll Key] ( ) . After the display “zeros,” remove the instrument cap from the chamber holder and remove the sample chamber.

5.4.9.1. This step “zeros” the meter while correcting for the color of the reagents. The colorimeter will turn on and the display will show "- - -" followed by "0.00".

5.4.10. After the instrument has been blanked, place each prepared sample cell into the cell holder in turn, covering with the instrument cap, and reading promptly to get an accurate result.

5.4.10.1. Press the [Read/Enter Key] (✓). The display will show "- - -" followed by the total ammonia measurement in mg/L.

5.4.11. Record the displayed reading in the *Parameter Check* logbook, LIMS/TOX_AMMONIA, and the appropriate bench sheets.

5.4.11.1. All readings measuring < 0.2 mg/L are recorded as < 0.2 mg/L.

5.4.11.2. Each sample MUST have a duplicate measurement (two cells used for each sample). However, if the sample replicate measurements vary by ≥0.02 mg/L, a third replicate must be

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measured. For replicates measuring ≤0.2, a third replicate is needed ONLY when one replicate is >0.2 and another is ≤0.2.

5.4.11.3. Average the measured values for each sample replicate to get the final value.

5.4.11.4. If an out-of-range indication is displayed (flashing "0.88"), dilute the sample with DI water. Be sure to record the dilution factor whenever a sample is diluted.

5.4.11.4.1. For example, if a sample is diluted in half (5 mL of sample and 5mL of DI water) the reported dilution factor would be 2X.

5.4.11.4.2. Because ammonia testing is for informational purposes only, samples above the readable range only need to be diluted once. If the sample is still above the readable range, it can be entered as ">0.88 with a dilution factor of X."

5.5. Perform steps in SECTION 5.4 to measure ammonia in the rest of the samples.

5.5.1. DI water is tested for ammonia weekly, the well water is tested for ammonia monthly. All bioassay/facility samples, dilution waters made in the lab, and received organism waters are tested for ammonia.

5.5.1.1. Organism water may have detectable ammonia. If the level of ammonia is significant, notify the lab supervisor immediately and note any dead organisms.

5.5.1.2. Moderately hard water, artificial saltwater (dilution water) and DI water should be ammonia free. If ammonia is detected in these waters, contact the lab supervisor immediately.

5.6. After all measurements have been taken, replace the cap, turn off the Colorimeter™ II by pressing the [Power/Backlight Key] (), and return the instrument to the carrying case. Rinse all sample cells (excluding SpecV™ Standards) with DI water. Turn them upside down and place in the drying rack. Clean up the work area.

6. DATA ARCHIVAL

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

6.2. Record information in Parameter Check logbook and Bioassay Bench Sheets logbooks.

6.3. FDEP Laboratory Information Management System (LIMS).

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6.4. 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.

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

6.4.2. 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.

6.4.3. 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.).

7. QUALITY CONTROL

7.1. Refer to Laboratory Quality Assurance 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 DEP Laboratory Quality Assurance Manual for sample disposal.

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

9. REFERENCES

9.1. HACH® Pocket Colorimeter™ Instruction Manual Nitrogen, Ammonia Method 8155

9.2. Standards Methods for the Examination of Water and Wastewater, 20th Edition: SM 4500-NH3

9.3. FDEP Laboratory Quality Assurance Manual

9.4. FDEP Health and Safety Manual

9.5. DEP SOP TA-06.32, Calibration and Verification of Mechanical and Disposable Pipettes in the Bioassay/AGP Lab

APPENDIX OF SIGNIFICANT CHANGES

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