# Protocol for measuring ammonia in air

## A. Sampling ammonia in air

### *Materials*

1. Aquarium air pump (Boyu Aquarium Air Pump 2 Output with Air Flow Controller).
2. Silicone tubing (2 nos.), 15 cm each, Inner diameter: 0.25 inch
3. 15 ml Falcon tubes, as required.
4. Test tube stand.
5. Aluminium foil.
6. Deionized water.

### *Procedure*

1. Place the air pump in the area where sampling is to be carried out.
2. Attach silicone tubings to the outlets in the air pump.
3. Add 5 ml deionized water to two 15 ml Falcon tubes.
4. Insert the silicone tubings in the Falcon tubes, such that they dip inside the water.
5. Set the air flow to ‘low’ using the controller present in the air pump
6. Cover the mouth of the tubes with aluminium foil to avoid loss of ammonia.
7. Switch on the air pump.
8. At the end of 15 min, remove one tube and cap it tightly. Label appropriately.
9. At the end of 30 min, remove the other tube and cap it tightly. Label appropriately.

*Notes*

1. Sampling time can be varied depending on ammonia in air.

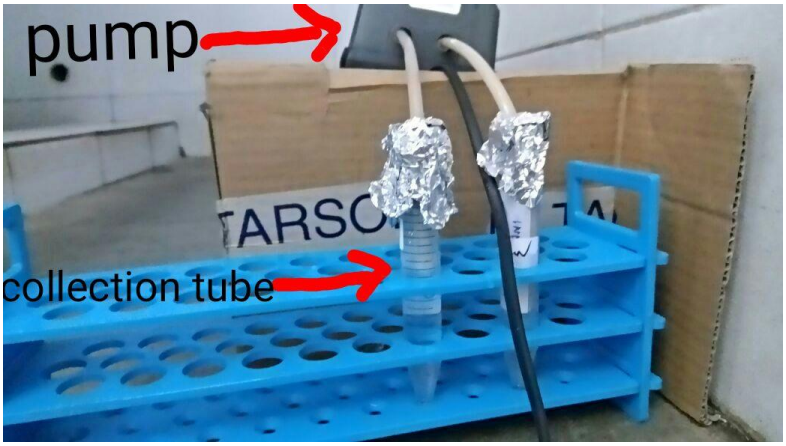


*Figure 1: Setup of the apparatus for sampling ammonia in air*

## B. Measuring ammonium

*(A new approach to indophenol blue method for determination of ammonium in geothermal waters with high mineral content, International Journal of Environmental Analytical Chemistry Volume 90, 2010 - Issue 2, pages 115 – 126)*

***Materials***

1. Phenol-ethanol solution: Add 1.1 ml liquified phenol (purity ≤ 89%) to 8.9 ml ethyl alcohol (95% v/v).
2. Sodium nitroprusside solution (0.5% w/v): Dissolve 0.05 g sodium nitroprusside in 10 ml deionized water.
3. Alkaline citrate solution: Dissolve 2 g trisodium citrate and 0.1 g sodium hydroxide in 10 ml deionized water.
4. Oxidizing solution: To 10 ml of alkaline citrate solution add 2.5 ml of 5% sodium hypochlorite to prepare the oxidising solution.
5. Ammonium chloride solution (1 mg/ml): Dissolve 0.01 g ammonium chloride in 10 ml deionized water.
6. Deionized water.

***Procedure***

1. Add 200 µl ammonium chloride solution to wells A1 and B1.
2. Add 100 µl distilled water in wells A2 to A12, B2 to B12, C1 to C12, D1 to D12 and E1, E2.
3. Transfer 100 µl of ammonium chloride solution from well A1 to A2.
4. Mix A2 well and transfer 100 µl to A3.
5. Mix A3 well and transfer 100 µl to A4.
6. Mix A4 well and transfer 100 µl to A5.
7. Mix A5 well and transfer 100 µl to A6.
8. Mix A6 well and transfer 100 µl to A7.
9. Mix A7 well and transfer 100 µl to A8.
10. Mix A8 well and transfer 100 µl to A9.
11. Mix A9 well and transfer 100 µl to A10.
12. Mix A10 well and transfer 100 µl to A11.
13. Mix A11 well and transfer 100 µl to A12.
14. Mix A12 well and transfer 100 µl to C1.
15. Mix C1 well and transfer 100 µl to C2.
16. Continue the dilution till C12. Discard 100 µl.
17. Repeat the same for wells B and D.
18. Do not continue dilution in wells E1 and E2.
19. Add 100 µl of samples to wells F, G and H (in triplicates).
20. Add 40 µL phenol-ethanol solution to each well. Mix well.
21. Add 40 µL sodium nitroprusside solution to each well. Mix well.
22. Add 100 µL oxidizing solution to each well. Mix well.
23. Incubate the plate for 20 min in the dark.
24. Record absorbance at 640 nm using a plate reader.
25. Import the data into a spreadsheet and plot a standard curve using measurements obtained from wells A to D that fall within the linear range.
26. Use the standard curve to calculate ammonium in samples.

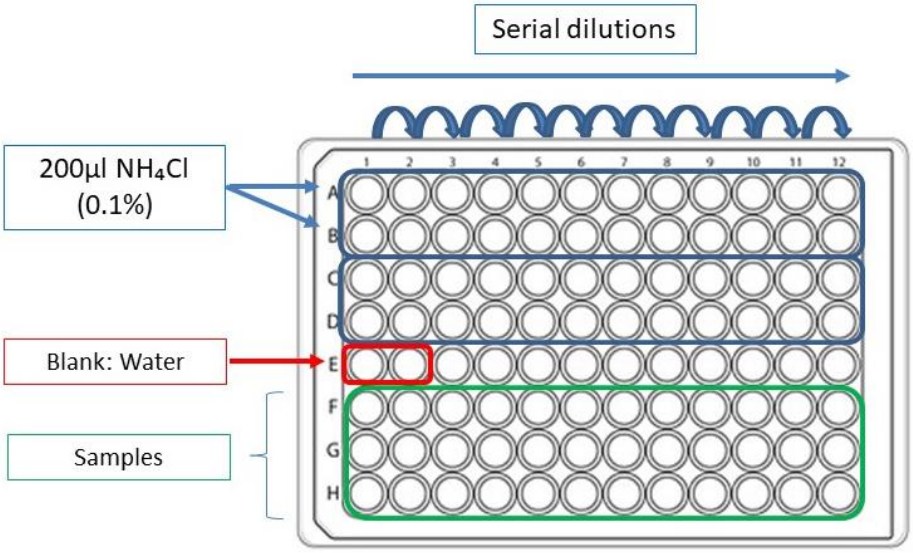


*Figure 2: Representative image of the plate layout*

*Notes*

1. The assay gives a linear response between ammonium chloride concentrations of 0.244 and 31.25 µg/ml.
2. Please dilute the samples if required.