

ALKEN-MURRAY CORPORATION	TITLE: STP PROCEDURE	NO. QC-2
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ALKEN-MURRAY CORPORATION

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QUALITY CONTROL METHOD - 2

STP Test

Description:

This procedure is a quality control test used to detect ammonia oxidation via a color change caused by a pH shift due to metabolic activity of *Nitrosomonas* and *Nitrobacter* bacteria, contained in certain **Alken Clear-Flo**[®] products (CF 1100, 1100-50xFF, 1400-50xFF, 7110 & 7110-50xFF). This test can be used at any time after manufacture to determine product viability after stress (exposure to extreme heat, freezing, seriously fluctuating temperatures, contamination, etc.). This procedure should be performed by a trained laboratory technician.

Equipment:

Spectrophotometer: visible wavelength range, bandpass 8nm or narrower, absorbance range 0 to 2, preferably absorbance is digitally displayed. (Alken-Murray uses a Perkin-Elmer model Lambda 2)

Incubator Shaker (such as made by New Brunswick) set at 34°C and 50-100 rpm

Adjustable air displacement pipettor (100 - 1000 µl) or two fixed pipettors at 100 µl and 1000 µl. See QC-1 for procedure to use this pipettor. Preferred brands: Eppendorf and Rainen.

A digital pH meter with pH and temperature probes (Alken-Murray uses Orion brand)

Magnetic stirrer

Drummond Pipet-Aid[®] with sterile 50 mL serological pipets or *Eppendorf* Repeater Pipettor with sterile 50 mL Combi-tips

Supplies:

As many as needed for testing, Sterile screw-cap test tubes (15 to 20 mL size)

Disposable cuvettes to test wavelength of solutions

Supply of pipet tips for pipettor

2 - 5 ml sterile serological pipet

2 - 10 ml sterile serological pipets

1 - 1 cc sterile syringe with 25 gauge needle (to adjust pH)

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- 100 ml bottle containing 40% solution of KHCO_3 or 20% solution of K_2CO_3
- 1 - 2 Liter (1000 ml) beaker for mixing
- 2 - 500 ml bottles with screw caps for storage of prepared STP solution (presterile)
- 8 - 100 ml sterile serological pipet
- 8 - Nalgene MF75 125 ml filter sterilizing units with 0.2 μm pores, with screw-cap sterile bottles for storage
- 2 - Nalgene MF75 500 ml filter sterilizing units with 0.2 μm pores, with screw-cap sterile bottles for storage

Ingredients of STP Medium

INGREDIENT	AMOUNT	AMC Lab Locations
Ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4]$	1.32 g	LS 1
Magnesium sulfate heptahydrate $[\text{MgSO}_4 \cdot 7\text{H}_2\text{O}]$	0.2 g	LS 1
Calcium chloride dihydrate $[\text{CaCl}_2 \cdot 2\text{H}_2\text{O}]$	0.02 g	LS 1
Dipotassium phosphate $[\text{K}_2\text{HPO}_4]$	0.087 g	SmD
EPPS buffer	2.52 g	SmD
Solution B	1.0 ml	Refrigerator
Solution C	1.0 ml	Refrigerator
Solution D	1.5 ml	Refrigerator
Distilled water	1.0 Liter	

Ingredients of Solution B (store refrigerated for up to 1 year, identical to Solution B in QC 53)

Sequestrene (chelated iron) OR Sigma EDTA - NaFe.....	100 mg
Distilled water.....	100 ml

Ingredients of Solution C (store individual components and complete mix for up to 1 year)

Sodium molybdate dihydrate $[\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}]$, 0.1% w/v.....	10 ml
Manganese sulfate monohydrate $[\text{MnSO}_4 \cdot \text{H}_2\text{O}]$, 0.1% w/v.....	17.2 ml
Cobalt chloride hexahydrate $[\text{CoCl}_2 \cdot 6\text{H}_2\text{O}]$, 0.1% w/v.....	0.4 ml
Zinc sulfate heptahydrate $[\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}]$, 0.1% w/v.....	10 ml
Distilled Water.....	to make 100 ml

Ingredients of Solution D (store at room temperature in the dark for up to 1 year)

Fisher-Scientific Phenol Red sodium salt.....	500 mg
Deionized or distilled H_2O	100 ml

Preparation of STP tubes:

1. Mix ingredients and adjust to volume.
2. Adjust pH to 7.8 with KHCO_3 (40% solution) or K_2CO_3 (20% solution) using approximately 25 drops/200 ml. Check this with spectrophotometer readings, as mentioned below.
3. Dispense approximately 9 ml into screw cap test tubes. We prefer to autoclave as we need these, rather than autoclaving the entire batch when made.
4. Autoclave for 15 minutes at 121°C (15 lbs. pressure) or filter sterilize with Nalgene MF75 500 ml filter sterilizing units with 0.2 μm pores. If autoclaved and cooling, the pH of the

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- solution should be between 7.6 and 7.85. This may need to be adjusted with acid, as necessary, to assure that the STP reagent is within this range.*
5. *Fill out appropriate information in Media Preparation QC database file. Sterilized tubes may be stored in the dark at room temperature for up to one year.*
 6. *Label all solutions and rack containing the STP tubes with initials, contents, preparation and expiration dates.*
 7. *If media was autoclaved, sacrifice one tube, after sterilizing to verify final pH of 7.8, with a spectrophotometer.*
 8. *As an alternative technique, adjust the solution to pH 7.8, after mixing and then filter sterilize it, using Nalgene MF75 filter-sterilizing units with 0.2 µm pores instead of autoclave. Fill screw-cap tubes with 9 mLs STP broth, dispensing from Eppendorf Repeater Pipettor with sterile 50 mL Combi-tips and label tubes.*
 9. *Store filled tubes in laboratory media refrigerator until needed by lab or to pack with client order of nitrifiers.*

Procedure:

1. *Inoculate 1 STP tube with 1 ml of CF 1100, 1100-50xFF, 1400-50XFF, 7110 or 7110-50xFF*
2. *Label tube with product, lot number, date and technicians initials (use LabPal to create vinyl labels.*
3. *Preferably, shake at 34° C temperature @ 75 to 120 rpm (faster if a lot of suspended matter in the tube from high concentrations of nitrifiers) with cap loosened to provide air. Alternatively, shake at lower temperature if 34° C incubator is not available, but qualify results by including estimated incubation temperature.*
4. *Check daily for color changes.*

Interpretation:

*Color should change to yellow within 7 days for 1X product at the time of manufacture, indicating metabolic activity (i.e. ammonia is oxidized by nitrifiers, pH is decreased, pH indicator detected pH change). Higher activity levels and viability will require less time for the STP test media to change color. Color should change within 24 hours for CF 1100-50xFF, 1400-50xFF or CF 7110-50xFF. A positive result can also be determined spectrophotometrically. **QC-96** provides instructions for calibration of your spectrophotometer for this purpose. For Alken-Murray's Perkin-Elmer Lambda 2 spectrophotometer, pH can be determined by dividing the absorbance measured at **558nm** by the absorbance at **480nm** and converting the resulting ratio, using the following chart of Ratio vs pH. If the pH has fallen from the starting value by 1.0 unit, the sample can be considered to have reached the endpoint of this test. If the pH has fallen by 0.5 units in two days, or less, the nitrifier product being tested can be considered acceptable for sale. Our experience shows that a pH drop of 0.5 in two days, or less, will result in a total of 1.0 units pH drop, in less than 7 days. Thus, a spectrophotometric assessment of a nitrifier sample can be made in less than half the time of the visual method. Record results in appropriate QC database log.*

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References:

Galen W. Ewing - Instrumental Methods of Chemical Analysis, McGraw Hill, 1969, pp.48 - 62.

Spectral Absorbance Ratio of $\frac{A_{558nm}}{A_{480nm}}$ vs pH

