HOWARD COLLEGE

SCHOOL OF CHEMICAL ENGINEERING

Pollution Research Group

STANDARD OPERATING PROCEDURES

2013

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The following SOP provides information on the shipping of samples and equipment to and from South Africa.

1. Courier into South Africa (University of KwaZulu-Natal)

The following procedure should be followed for the importing of chemicals / equipment / samples into South Africa to the University of KwaZulu-Natal (Pollution Research Group):

- Contact the courier to be used and request advice on the correct forms to use and how to complete these correctly to avoid problems with the South African Customs and inappropriate import duties.
- Equipment in particular must be marked for research purposes and the forms must be completed correctly.
- Visitors will need to cover any customs charges, unless an arrangement has been made with PRG to cover the costs.
- International courier companies sending goods from abroad will generally use their South African branch to clear the goods. The South African branch will then contact the PRG to determine who will pay any duties/import charges, and to confirm a delivery day (it is therefore important that the sender from abroad has the correct contact and delivery details for PRG).
- Generally the duties are paid on delivery and a credit card needs to be used for this. The sender needs to make an arrangement with PRG if they are not going to be here when the goods arrive. Not all goods have duties on them so there may be no cost.
- An arrangement can be made with UKZN Registry to clear the good should the import costs be high. This will then be charged to a PRG cost centre. If PRG are not covering the costs arrangements will need to be made to repay the cost centre.

The most important aspects to remember are:

1. The Courier company needs to make sure the paperwork is completely correctly and that the sender has communicated clearly the reason why the goods are being couriered
2. The sender/visitor needs to ensure an arrangement has been made should there be duties to be paid once the goods arrive in South Africa.

2. Courier from South Africa (University of KwaZulu-Natal)

Any courier can be used for sending samples / equipment from South Africa, and the same guidelines apply as listed above. If the University system is to be used, the courier details and procedure are provided below.

University Courier information

- The University courier(as at July 2013, check annually for changes) is **Globeflight** –telephone number +27 (0)31 242 3000.

- They can be used to send goods/docs from the University or they can collect goods/docs from another point, locally or internationally.

General procedure for using Globeflight

- A call is placed to Globeflight to book the pickup. The following information will be requested:
  - Collection address
  - Contact person and telephone number
  - PRG account number (**D11544**)
  - The cost centre code to be charged (available from PRG)
  - The General Ledger Code (GLA) - **30061 (courier)**
  - Destination of delivery

- The anticipated cost of a courier service can be provided by a quote from Globeflight. This can be requested via email. Call Globeflight and request an email address.

3. Contact details for the Pollution Research Group

Kerry Lee Philp
Tel: +27 (0)31 260 3375 (landline)
Cell: +27 (0)72425 2741 (cell/mobile)

Physical address	**Postal address**
Pollution Research Group Basement, New Chemical Engineering Building King George V Avenue (Entrance 3, off Rick Turner Road) University of KwaZulu-Natal Howard College Campus Glenwood Durban	Pollution Research Group Chemical Engineering University of KwaZulu-Natal Durban 4041 SOUTH AFRICA

PPROVAL OF STANDARD OPERATING PROCEDURE

PRG Head: Prof Buckley

Signature: ……

Date: ……

Author Merlien Reddy:

Signature: ……

Date: ……..

Standard Operation Procedure – Nitrogen, Ammonia

1. Scope and Application

- Ammonia is naturally is present in surface and wastewaters.
- It is produced largely by the deamination of waters and wastewaters the forms of nitrogen of the greatest interest are, in order of decreasing oxidation state, nitrate, nitrite, ammonia and organic nitrogen.
- All these forms of nitrogen as well as nitrogen gas are biochemically interconvertible and are components of the nitrogen cycle.
- The method covers the range from about 10 to 25mg/L for titrimetric procedure.

2. Summary

- The sample is buffered at pH 9.5 with a borate buffer to decrease hydrolysis of cyanates and organic nitrogen compounds.
- It is distilled into a solution of boric acid when titration is to be used.
- The ammonia in the distillate is determined titrimetrically with standard sulphuric acid and a mixed indicator together with a pH meter.

3. Interferences

- Glycine, urea, glutamic acid, cyanates and acetamide hydrolyze very slowly in solution on standing but of these only urea and cyanates hydrolyze on distillation at pH 9.5.

4. Sampling

- Most reliable results are obtained on fresh samples
- Destroy residual chlorine immediately after sample collection to prevent its reaction with ammonia.
- If an immediate analysis is not possible, preserve samples by acidifying to pH between 1.5 and 2.0 with 0.8 ml conc H_2SO_4/L and store at 4ºC.
- If acid preservation is used, neutralize samples with NaOH or KOH immediately before making the determination.

5. Safety Precautions

- Always use safety goggles, gloves and laboratory coat while working in laboratory
- Use eye and hand protection when preparing acid or handling color reagent
- Prepare and keep color reagent in fume hood

6. Apparatus

- VELP Distillation unit
- HANNA pH meter

7. Reagents

- **Ammonia Free Water**

Eliminate traces of ammonia in distilled water by adding 0.1ml sulphuric acid to 1L distilled water and redistilled. Alternately treat distilled water with enough bromine or chlorine water to produce a free halogen residue of 2-5 mg/L and redistill after standing for 1 hr.

- **0.1N NaOH**

Dissolve 4g NaOH in 1L distilled water.

- **1N NaOH**

Dissolve 40g NaOH in 1 ammonia free distilled ater.

- **Borate Buffer Solution**

Add 88mL of 0.1N NaOH solution to 500ml of 0.025M di-sodium tetra borate-hydrous ($Na_2B_4O_7$. $10H_2O$) solution – (9.5g $Na_2B_4O_7$. $10H_2O$ hydrous per liter) or (5.0g $Na_2B_4O_7$ anhydrous per liter) and dilute to 1L.

- **Mixed indicator Solution**

Dissolve 200mg methyl red indicator in 100ml 95% ethyl or isopropyl alcohol or ethanol. Dissolve 100mg methylene blue in 50ml 95% ethyl or isopropyl alcohol or ethanol. Combine solutions. Prepare monthly.

- **Indicating Boric acid Solution**

Dissolve 20g H_3BO_3 in ammonia free distilled water, add 10ml mixed indicator solution and dilute to 1L. Prepare monthly.

- **Standard Sulphuric acid Titrant, 0.02N**

Dissolve 0.5ml conc sulphuric acid in distilled water and dilute to 1liter.

Weigh out about 1.325g anhydrous Sodium Carbonate, previously dried at 270 ºC. Dissolve in distilled water and make up to 250ml in a volumetric flask- this is 0.10N. Do not keep longer than 1 week.

Titrate the sulphuric acid solution against 25ml of sodium carbonate solution using bromocresol green-methyl red mixed indicator. Calculate the normality of the sulphuric acid.

Error! Bookmark not defined.$Normailty\ of\ H_2SO_4\ Solution = \frac{25 \times 0.1}{Vol\ H_2SO_4\ used}$

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8. Sample Preparation – Fecal Sludge

- Weigh out between 1.8g and 2g of well mixed fecal sludge sample.
- Place the weighed out sample into a blender with 250ml of distilled water.
- Blend for 30 seconds.
- Transfer the blended mixture into a volumetric flask and top up to 1L with distilled water.
- Transfer the 1L solution to a plastic bottle and store in the cold room.

9. Procedure

- Preparation of Equipment

Add 500ml ammonia – free water and 20 ml borate buffer to a distillation flask and adjust pH to 9.5 with 6N NaOH solution. Add a few glass beads and use this mixture to steam out the distillation apparatus until distillate shows no traces of ammonia.

Ammonia Nitrogen In Sample Mg/L	Sample Volume mL
5-10	250
10-20	100
20-50	50.0
50-100	25.0

- Add 70ml of sample to distillation flask.
- Add 20ml borate buffer to distillation flask.
- Distill for 5min and collect 100ml distillate into the 50 ml indicating boric acid solution.
- Titrate ammonia in distillate with standard 0.02N sulphuric acid, titrate until indicator turns a pale lavender.
- Carry a blank through all steps of the procedure and apply the necessary correction to the results.

Calculation:

$$NH_3\ (mg/L) = \frac{(A - B) \times 280}{Sample\ (ml)}$$

Where:

A = volume of H_2SO_4, titrated for sample, ml

B = Volume of H_2SO_4, titrated for blank, ml

Sulphuric acid : Standard solution(0.02N, 1mL=0.28mg NH3-N) 1L-280mg NH3- N

Concentration = mass/Molar mass

$$NH_3\ in\ Wet\ Sample\ (mg/g) = \frac{(A - B) \times 280}{Sample\ (ml)} \times \frac{V}{M}$$

$$NH_3\ in\ Wet\ Sample\ (g/g) = \frac{NH_3\ in\ Wet\ Sample\ (mg/g)}{1000}$$

$$NH_3\ in\ Dry\ Sample\ (g/g) = \frac{NH_3\ in\ Wet\ Sample\ (g/g)}{Total\ Solids\ (g/g)}$$

Where:

M = mass of sludge used in sample preparation (g)

V = Volume of dilution (L)

Quality control:

- **Ammonium chloride, stock solution: 1.0mL=1.0mg NH3-N. Dissolve 3.819g NH_4Cl in distilled water and bring volume to 1L with distilled water in a volumetric flask.**
- **Ammonium chloride, STD solution: 1.0mL = 0.01mg. Dilute 10.0mL of stock solution to 1Liter in a volumetric flask to give a concentration of 10mg/L NH_3-N.**

10. Precision and Accuracy

mg NH3-N/L %SD %Error

APPROVAL OF STANDARD OPERATING PROCEDURE

PRG Head: Prof Buckley Signature: ……

Date: ……

Author Merlien Reddy: Signature: ……

Date: ……..

Standard Operation Procedure – Chemical Oxygen Demand Closed Reflux, Titrimetric Method

1. Scope and Application

- The Chemical Oxygen Demand (C.O.D) measures the oxygen equivalent of that portion of the organic matter in a sample that is easily oxidized by a strong chemical oxidant.
- It is an important and rapidly measured parameter to measure the amount of organic compounds in stream and industrial waste studies, and in operational control of waste water treatment plants. It is also applicable for measurements on human excreta.
- This procedure described hereafter is applicable to COD values 40-400mg/L.

2. Summary

The sample is digested for 2hours in a strongly acidic dichromate solution, using silver sulphate as a catalyst and mercuric sulphate as a masking agent to prevent chloride interference. The dichromate is partially reduced by the oxidizable material present in the sample. The excess dichromate is titrated with ammonium iron (II) sulphate and the COD value calculated from the amount of dichromate.

The half reaction for the reduction of dichromate is:

$Cr_2O_7^{2-} + 14H^+ + 6e^- \rightarrow 2Cr^{3-} + 7H_2O$

The remaining dichromate is titrated with a standard ammonium iron(II) sulphate solution:

$Cr_2O_7^{2-} + 6Fe^{2+} + 14H^+ \rightarrow 6Fe^{3+} + 7H_2O + 2Cr^{3+}$

The equivalence point is indicated by the sharp colour change from blue-green to red as the ferroin indicator undergoes reduction from iron (III) to the iron (II) complex.

3. Interferences

- Difficulties caused by the presence of chlorides in the sample are overcome by the addition of mercuric sulphate to samples before digesting. The chloride ion is then eliminated from the reaction by forming a soluble mercuric chloride complex.

- A catalyst must be used to include some organic compounds (e.g. acetic acid), while other biological compounds (eg cellulose), which are not important, are included in the determination. Pryridine is not oxidized even in the presence of the catalyst.

4. Sampling

- Preferably collect samples in glass bottles.
- Test unstable samples without delay.
- Preserve samples by acidifying with concentrated sulphuric acid to pH 2.
- Determine COD on well shaken samples. Settled samples may also be analysed if requested.
- 5ml pipette to measure out sample.

5. Safety Precautions

- Handle concentrated sulphuric acid with care.
- Always use safety goggles, gloves and laboratory coat while working in laboratory
- Wear face shield and protect hands from heat produced when contents of the vessels are mixed.
- After the analysis clean bottles and beakers with water then dry
- Dispose the used gloves after completion of analysis

- Clean the hands using antiseptic soap
- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.

6. Apparatus

- Carousal of 10 teflon vessels
- 100 ml Erlenmeyer flasks
- 5ml pipette
- 10ml and 5ml automatic bottle top dispensers

7. Sample Preparation –Fecal Sludge

- Weigh out between 1.8g and 2g of well mixed fecal sludge sample.
- Place the weighed out sample into a blender with 250ml of distilled water.
- Blend for 30 seconds.
- Transfer the blended mixture into a volumetric flask and top up to 1L with distilled water.
- Transfer the 1L solution to a plastic bottle and store in the cold room.

8. Reagents

Standard Potassium Dichromate $K_2Cr_6O_7$ Digestion Solution: 0.0167M

Add to about 500ml distilled water 4.913g $K_2Cr_6O_7$, previously dried at 105 °C for 2hrs.

Add 167ml concentrated Sulphuric acid H_2SO_4 and 13.3g Mercuric Sulphate HgSO4.

Dissolve and cool to room temperature before diluting to 1L.

Sulphuric Acid H_2SO_4 /Silver Sulphate Reagent Ag_2SO_4 (COD Reagent)

Add **26g of silver sulphate** crystals or powder to 2.5L of concentrated sulphuric acid using a magnetic stirrer. Shake well and leave for 2days for dissolution.

Ferroin Indicator 2 drops

Dissolve 1.485g 1:10 phenentroline monohydrate and 0.695g ferrous sulphate ($FeSO_4.7H_2O$) in distilled water and dilute to100ml.

Ferrous Ammonium Sulphate Fe $(NH_4)_2$ $(SO_4)_2.6H_2O$: 0.10M

Dissolve 39.2g Fe $(NH_4)_2$ $(SO_4)_2.6H_2O$ in distilled water.

Add 20ml concentrated Sulphuric acid H_2SO_4 and dilute to 1L.

Standardize daily against **Standard Potassium Dichromate $K_2Cr_6O_7$ Digestion Solution**

9. Calibration

- Prepare a standard $K_2Cr_2O_7$ solution daily to correct any variation in the concentration of the Ferrous Ammonium Sulphate.
- Prepare a blank with each set of samples consisting of 5 ml distilled water in place of sample together with all the reagents and digest together with samples.
- **Standard Preparation**
- Add 3ml of standard $K_2Cr_2O_7$ digestion solution to 5 ml of distilled water. Add 7ml COD reagent and cool it down. Titrate with FAS titrant using 2 drops of ferroin indicator.
- **Quality Control: Potassium hydrogen Phthalate (KHP)**
  Lightly crush and then dry out KHP to a constant weight at 120°C. Dissolve 0.0425g in distilled water and then dilute to 250ml. This solution has a theoretical COD of 200mg/L. Solution is stable if refrigerated, for a period of 3 months in the absence of biological growth.

10. Procedure

Sample Preparation

- Add 5ml sample to each teflon vessel.
- Add 5ml distilled water to another vessel (blank).
- Add 3ml potassium dichromate digestion solution into each vessel.
- Add 7ml sulphuric acid reagent (with silver sulphate) in each vessel.
- The acid must be poured down the wall of the flask while flask is tilted. If sample is too concentrated it will turn green, and a higher dilution of sample must be used.

Digestion

- Place teflon vessels into the rotor, with the temperature probe placed into the teflon vessel labeled 1.
- Switch on the microwave and select COD METHOD:
- 15min ramping time to 150 °C, 30min digestion at 150°C and 1hr cooling to 50 °C.
- Transfer contents from teflon vessels into 100ml flasks for titrating.

Titration

- Titrate the excess dichromate in the digest mixture with standard ferrous ammonium sulphate using 2 drops of ferroin indicator.
- Titrate from a sharp green/orange to red brown end point.
- Take reading.**Error! Bookmark not defined.**

Calculation

$$COD\ (mg\ O_2/L) = \frac{(Blank - Titration)\ \times molarity\ of\ FAS\ \times 8000}{Sample\ (ml)}$$

Where:
8000 = milliequivalent weight of oxygen × 1000 ml/L

$$Molarity\ of\ FAS = \frac{Volume\ 0.0167M\ K_2Cr_2O_7\ Solution\ Titrated\ (ml)}{Volume\ FAS\ used\ in\ titration\ (ml)} \times 0.10$$

$$COD\ (mg\ O_2/L) = \frac{(Blank - Titration)\ \times molarity\ of\ FAS\ \times 8000}{Sample\ (ml)} \times \frac{V}{M}$$

$$COD\ in\ Wet\ Sample\ (g\ O_2/g) = \frac{COD\ (mg\ O_2/L)}{1000}$$

$$COD\ in\ Dry\ Sample\ (g\ O_2/g) = \frac{COD\ in\ Wet\ Sample\ (g\ O_2/g)}{Total\ Solids\ (g/g)}$$

Where:

V = Total volume (L)

M = Mass of sludge used in sample preparation (g)

11. Precision and Accuracy

mg COD/L %SD %Error

APPROVAL OF STANDARD OPERATING PROCEDURE

PRG Head: Prof Buckley

Signature: ……

Date: ……

Author Merlien Reddy:

Signature: ……

Date: ……..

Standard Operation Procedure – Nitrate Electrode Method

1. Selection of Method

Total oxidized nitrogen is the nitrate plus nitrite nitrogen. Nitrate generally occurs in trace quantities in surface water but may attain high levels in some groundwater. In excessive amounts, it contributes to the illness known as methemoglobinemia. Determination of nitrate is difficult because of the relatively complex procedures required, the high probability that interfering constituents will be present, and the limited concentration ranges of the various techniques. Application range for nitrate electrode method includes 0.14 to 1400mg NO_3^- -N/L.

2. Principle

The NO_3^- ion electrode is a selective sensor that develops a potential across a thin, porous, inert membrane that holds in place a water-immiscible liquid ion exchanger. The electrode responds to NO_3^- ion activity between 10^{-5} and 10^{-1} *M* (0.14 to 1400 mg NO_3^- -N/L). The lower limit of detection is determined by the small but finite solubility of the liquid ion exchanger.

3. Interferences

- Chloride and bicarbonate ions interfere when their weight ratios to NO_3^- -N are >10 or >5, respectively. Ions that are potential interferences but do not normally occur at significant levels in portable waters are NO^{2-}, CN^-, S^{2-}, Br^-, I^-, ClO^{3-}, and ClO^{4-}. Although

the electrodes function satisfactory in buffers over the range of pH 3 to 9, erratic responses have been noted where pH is not held constant. Because the electrode responds to NO_3^- activity rather than concentration, ionic strength must be constant in all samples and standards. Minimize this problem by using a buffer solution containing Ag_2SO_4 to remove Cl^- , S^{2-}, Br^-, I^-, and CN, sulfamic acid to remove NO_2^-, a buffer at pH 3 to eliminate HCO_3^- and to maintain a constant pH and ionic strength, and $Al_2(SO4)3$ to complex organic acids.

4. Sampling

- Start nitrate determinations promptly after sampling. If storage is necessary, store up to 2days at 4ºC, disinfected samples are stable for much longer without acid preservation. For longer storage of unchlorinated samples, preserve with 2ml concentrated H_2SO_4/L and store at 4 ºC. When sample is preserved with acid nitrite and nitrate cannot be determined as individual species.

5. Safety Precautions

- Always use safety goggles, gloves and laboratory coat while working in laboratory
- Use eye and hand protection when preparing acid or handling color reagent
- Prepare and keep color reagent in fume hood

6. Apparatus

- PH meter, expanded scale or digital, capable of 0.1 mV resolution.

- Double junction reference electrode (Orion model 90-02 or equivalent) Fill outer chamber with $(NH_4)_2SO_4$ solution.
- Nitrate ion electrode (Orion model 93-07, Corning model 476134 or equivalent). Carefully follow manufactures instructions regarding care and storage.
- Magnetic stirrer: TFE- coated stirring bar.

7. Reagents

- **Stock Nitrate Solution**

Dry potassium nitrate (KNO_3) in an oven at 105ºC for 24hr. Dissolve 0.7218g in water and dilute to 1000ml, 1.00ml = 100ug NO_3^- -N. Preserve with 2ml $CHCl_3$/L. This solution is stable for at least 6 months.

- **Standard Nitrate Solution**

Dilute 1.0, 10 and 50ml stock nitrate solution to 100ml with water to obtain standard solutions of 1.0, 10, and 50 mg of NO_3^- -N/L respectively.

- **Buffer solution**

Dissolve 17.32g $Al_2(SO_4)_3$.18 H2O, 3.43g Ag_2SO_4, 1.28g H_3BO_3 and 2.52g sulfamic acid(H_2NSO_3H) in about 800ml water. Adjust to pH 3 by slowly adding 1N NaOH. Dilute to 1000ml and store in a dark bottle.

- **Sodium Hydroxide**

NaOH 1N

- **Reference Electrode filling solution**

Dissolve 0.53g $(NH_4)_2SO_4$ in water and dilute to 100ml.

8. Calibration

Transfer 10ml of 1mg NO_3^- -N/L standard to a 50 ml beaker, add 10 ml buffer and stir with a

magnetic stirrer. Immerse tips of electrodes and record millivolt reading when stable (after 1 min). Remove electrodes, rinse and blot dry. Repeat for 10-mg NO_3^- -N/L and 50 mg NO_3^- -N/L standards. Plot potential measurements(X axis- in millivolts) against NO_3^- -N concentration (Y). A straight line with a slope of +57 ±3 mV/decade at 25ºC should result. Recalibrate electrodes several times daily by checking potential readings of the 10mg NO_3^- -N standard and adjusting the calibration control until the reading plotted on the calibration curve is displayed again.

9. Procedure

- Transfer 10ml sample to a 50ml beaker, add 10ml buffer solution and stir (1min) with a magnetic stirrer. Measure standards and samples at about the same temperature. Immerse electrode tips in sample and record potential readings when stable (1min).

Calculation

- Prepare a standard curve by plotting potential measurements(X axis- in millivolts) against NO_3^--N concentration (Y). Compute sample concentration directly from curve.

Standard Operation Procedure – Nitrite

1. Scope and Field of Application

In water and wastewater, Nitrite is an intermediate oxidation state of nitrogen, both in the oxidation of ammonia to nitrate and in the reduction of nitrate. Such oxidation and reduction may occur in wastewater treatment plants, water distribution systems and natural waters.

2. Principle

Nitrite (NO^{2-}) is determined through the formation of a reddish purple azo dye produced at pH 20. to 2.5 by coupling diazotized sulfanilamide with N-(1-Naphthyl)-ethylenediamine dihydrochloride.

The application range of the method for spectrophotometric measurements is 10 to 1000 ug NO^{2-} -N/L. The colour system obeys Beer's law up to 180ug N/L with a 1cm light path at 543nm. Higher nitrite concentrations can be determined by diluting a sample

3. Interferences

- The following ions interfere because of precipitation under test conditions and should be absent:Sb^{3+} , Au^{3+} , Bi^{3+}, Fe^{3+} , Pb^{2+} , Hg^{2+} , Ag^{2+} , chloroplatinate($PtCl_6^{2-}$) , and metavanadate (Va_3^{2-}). If samples are suspected to contain heavy metals, 0.5 % EDTA solution can be added.

- Colored ions that alter the colour system also should be absent. Remove suspected solids by filtration.

4. Sampling

- Collect samples in glass bottles
- Analyze samples as soon as possible(within 24hrs of collection)
- Store those samples at 2-5ºC which may be preserved subject to verification.

5. Safety Precautions

- Always use safety goggles, gloves and laboratory coat while working in laboratory
- Use eye and hand protection when preparing acid or handling color reagent
- Prepare and keep color reagent in fume hood

6. Apparatus

- Laboratory glassware cleaned with 2M hydrochloric acid and rinsed thoroughly and distilled water
- Spectrophotometer, with a wavelength of 543 nm, together with cells of optical path length 1cm

7. Reagents

- **Nitrite Colour Reagent**

To 800ml distilled water, add 100ml 85%Phosphoric acid and 10g Sulphanilamide. Dissolve completely then add 1g N-(1-naphthyl)-ethylenediamine dihydrochloride. Dilute to 1L with water then store in a dark bottle in fridge for 1 month only.

- **Stock Sodium Nitrite**

Dissolve 1.232g $NaNo_2$ (anhydrous) in 1L distilled water. 1ml = 250 ug N

- **Standard Sodium Nitrite**

Dissolve 10ml stock solution in 1L distilled water. 1ml = 2.5 ug N

8. Calibration

Into each of 4 separate 50 ml volumetric flasks add 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml of standard $NaNO_2$. These are equivalent to 2.5, 5.0, 7.5 and 10 ug of N_2 respectively. Add colour reagent as per sample and read on a spectrophotometer at 543nm.

9. Procedure

- *Removal of suspended solids*: If samples contain suspended solids, filter through a 0.45um-pore-diam membrane filter.
- *Colour Development*: If sample pH is not between 5 and 9 adjust to that range with 1*N* HCL or NH_4OH as required.

- Pour 20ml sample into a 100ml volumetric flask.
- Add 2ml nitrite reagent.
- Bring to 100ml with distilled water.
- *Photometric measure*: Between 10 an 2hr after adding color reagent to samples and standards, measure the absorbance at 543nm.
- Nitrite levels are too high when samples turn pink.

Calculation

- Prepare a standard curve by plotting absorbance of standards against NO_2^- -N concentration. Compute sample concentration directly from curve.

Standard Operation Procedure – pH of Faecal Sludge

1. Scope and Field of Application

This method is an electrometric procedure for measuring pH in soils and waste samples. Wastes may be solids, sludges, or non-aqueous liquids. If water is present, it must constitute less than 20% of the total volume of the sample.

2. Interferences

Samples with very low or very high pH may give incorrect readings on the meter. For samples with a true pH of >10, the measured pH may be incorrectly low. This error can be minimized by using a low-sodium-error electrode. Strong acid solutions, with a true pH of <1, may give incorrectly high pH measurements.

Errors will occur when the electrodes become coated. If an electrode becomes coated with an oily material that will not rinse free, the electrode can (1) be cleaned with an ultrasonic bath, or (2) be washed with detergent, rinsed several times with water, placed in 1:10 HCl so that the lower third of the electrode is submerged, and then thoroughly rinsed with water, or (3) be cleaned per the manufacturer's instructions.

3. Safety Precautions

- Always use safety goggles, gloves and laboratory coat while working in laboratory
- After the analysis clean bottles and beakers with clear water keep it for drying
- Dispose the used gloves after completion of analysis
- Clean the hands using antiseptic soap
- Disinfect hands after washing with soap

- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.

4. Apparatus

- pH meter with means for temperature compensation.
- Glass electrode.
- Reference electrode -- A silver-silver chloride or other reference electrode of constant potential may be used.
- 50 ml beaker
- Thermometer and/or temperature sensor for automatic compensation.
- Analytical balance -- capable of weighing 0.1 g.

5. Procedure

Sample Preparation

- To 20 g of waste sample in a 50 ml beaker, add 20 ml of distilled water, cover, and continuously stir the suspension for 5 min. Additional dilutions are allowed if working with hygroscopic wastes and salts or other problematic matrices.
- Let the waste suspension stand for about 15 min to allow most of the suspended waste to settle out from the suspension or filter or centrifuge off aqueous phase for pH measurement.

NOTE: If the waste is hygroscopic and absorbs all the reagent water, begin the experiment again using 20 g of waste and 40 ml of reagent water.

NOTE: If the supernatant is multiphasic, decant the oily phase and measure the pH of the aqueous phase. The electrode may need to be cleaned if it becomes coated with an oily material.

Measurement of pH

Adjust the electrodes in the clamps of the electrode holder so that, upon lowering the electrodes into the beaker, the glass electrode will be immersed just deep enough into the clear supernatant to establish good electrical contact through the ground glass joint or the fiber-capillary hole.

Insert the electrode into the sample solution in this manner. For combination electrodes, immerse just below the suspension.

If the sample temperature differs by more than 2 °C from the buffer solution, the measured pH values must be corrected.

6. Results

Report the results as "waste pH measured in water at __°C" where "__°C" is the temperature at which the test was conducted.

7. References

http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/9045d.pdf

Standard Operation Procedure – Potassium Cell Test (Cat. No. 1.14562)

1. Scope and Field of Application

Measuring range 5.0 – 50 mg/l K

2. Principle

In alkaline solution, potassium ions react with Kalignost (sodium tetraphenyl-borate) to form a slightly soluble precipitate. The resulting turbidity is measured in the photometer (turbidimetric method)

3. Sampling

- Analyze immediately after sampling
- Use the Merckoquant Ammonium Test to check ammonium content. Samples containing more than 50mg/l NH_4^+ (Cat. No. 114562) or 150mg/l NH_4^+ (Cat. No. 100615) must be diluted with distilled water
- Check the potassium content with a Merckoquant Potassium Test. Samples containing more than 50 mg/l K (Cat. No. 114562) or 300 mg/l K (Cat. No. 100615) must be diluted with distilled water
- The pH must be within the range 3 – 12, if necessary, adjust with NaOH (1 mol/l) or H_2SO_4 (0.5 mol/l)
- Turbid sampled must be filtered

4. Safety Precautions

- Handle concentrated sulphuric acid with care.
- Always use safety goggles, gloves and laboratory coat while working in laboratory
- Wear face shield and protect hands from heat produced when contents of the vessels are mixed.
- After the analysis clean bottles and beakers with water then dry
- Dispose the used gloves after completion of analysis
- Clean the hands using antiseptic soap
- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.
- Reclose the reagent bottles immediately after use
- **The test reagents must not be run off with the wastewater**

5. Apparatus

- SQ118 Photometer

6. Reagents

- Reagent K-1K – Formaldehyde (≥25% - <50%); Methanol (≥10% - <20%)
- Reagent K-2K – Sodium tetraphenylborate (<100%)

Other required reagents and accessories

- Merckoquant Ammonium test (Cat. No. 110024)
- Merckoquant Potassium test (Cat. No. 110042)
- Universal indicator strips pH 0 – 14 (Cat. No. 109535)
- Alkalit indicator strips pH 7.5 – 14 (Cat. No. 109532)
- Potassium standard solution CertiPUR, 1000 mg/l K (Cat. No. 170230)

7. Sample Preparation

Fecal samples are diluted by blending 1.8g -2g sample into 1L of distilled water, as described in detail below:

- Weigh out 1.8g – 2g fecal sample using an analytical balance and add to a blender with 100mL distilled water and blend.
- Add blended sample to a 1L volumetric flask and dilute to 1L using distilled water.
- Swirl flask until sample is completely dissolved.

Filtration

Filter paper dimensions: diameter = 47mm, pore size = 0.45 microns

- Filter the diluted solution using a Buchner funnel.
- Collect the filtrate for analysis.

7. Procedure

	114562	
Pretreated Sample **(20 - 30°C)**	2.0 ml	Pipette into a reaction cell **(20 - 30°C)**, close the cell and mix. **The pH must be within the range 10.0 to 11.5.** Check with Alkalit Indicator strips. Adjust the pH if necessary with NaOH solution (1 mol / l)
Reagent K-1K	6 drops	Add and mix

Reagent K-2K	1 level blue microspoon (in the cap of the K-2K bottle)	Add and dissolve by shaking the tightly closed cell
Leave to stand for exactly 5 min (reaction time), then measure the sample in the photometer		

Note on the measurement:

- For turbidi-metric measurement, the cells must be clean. Wipe if necessary with a clean dry cloth
- The turbidity of the measurement solution remains stable for only a short time (the measurement value increases by 5-7% per minute)

9. Disposal of Waste Chemicals

- Collect waste in a labeled 2.5L bottle for collection from Waste Tech.

10. Calculations

$$Wet\ Sample\ Concentration\ (g/g) = \frac{A}{1000} \times \frac{V}{M}$$

$$Dry\ Sample\ Concentration\ (g/g) = \frac{Wet\ Sample\ Conc.\ (g/g)}{Total\ Solids\ (g/g)}$$

Where:

$A - Spectroquant\ Reading\ Concentration$
$V - Volume\ of\ Dilution\ (L)$
$M - Mass\ of\ Sludge\ used\ in\ sample\ preperation\ (g)$

Standard Operation Procedure – Sodium Cell Test (Cat. No. 1.00885.0001)

1. Scope and Field of Application

Measuring range 10 – 300 mg/l Na

2. Principle

The chloride ions equivalent to the sodium ions react with mercury(II) thiocyanate to form slightly dissociated mercury(II) chloride. The thiocyanate released in the process in turn reacts with iron(III) ions to form red iron(III) thiocyanate that is determined photometrically.

3. Sampling

- Analyze immediately after sampling
- Turbid sampled must be filtered

4. Safety Precautions

- Always use safety goggles, gloves and laboratory coat while working in laboratory
- After the analysis clean bottles and beakers with water then dry
- Dispose the used gloves after completion of analysis
- Clean the hands using antiseptic soap
- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.
- Reclose the reagent bottles immediately after use

- **The test reagents must not be run off with the wastewater**

5. Apparatus

- SQ118 Photometer

6. Reagents

- Na- 1K – Methanol (≥ 1% - < 3%); Mercury(II)thiocyanate (≥ 1% - <2.5%)

Other required reagents and accessories

- Universal indicator strips pH 0 – 14 (Cat. No. 109535)
- Chloride standard solution CertiPUR, 1000 mg/l Cl (Cat. No. 119897)

7. Sample Preparation

Faecal samples are diluted by blending 1.8g -2g sample into 1L of distilled water, as described in detail below:

- Weigh out 1.8g – 2g faecal sample using an analytical balance and add to a blender with 100mL distilled water and blend.
- Add blended sample to a 1L volumetric flask and dilute to 1L using distilled water.
- Swirl flask until sample is completely dissolved.

Filtration

Filter paper dimensions: diameter = 47mm, pore size = 0.45 microns

- Filter the diluted solution using a Buchner funnel.
- Collect the filtrate for analysis.

8. Procedure

	100885	
Reagent Na-1K	0.50 ml	Pipette into a reaction cell, close the cell and mix.
Pretreated Sample **(10 - 30°C)**	0.50 ml	Add with pipette, close the cell, and mix.
Leave to stand for exactly 1 min (reaction time), then measure the sample in the photometer		

Note on the measurement:

- For photometric measurement, the cells must be clean. Wipe if necessary with a clean dry cloth
- Measurement of turbid solutions yields false-high readings.
- The pH of the measurement solution must be approx. 1.
- The colour of the measurement solution remains stable for 30 min after the end of the reaction time stated above.

9. Disposal of Waste Chemicals

- Dilute 10 ml into 1000ml.
- Flush down the sink with excess water.

10. Calculations

$$Wet\ Sample\ Concentration\ (g/g)\ = \frac{A}{1000} \times \frac{V}{M}$$

$$Dry\ Sample\ Concentration\ (g/g)\ = \frac{Wet\ Sample\ Conc.\ (g/g)}{Total\ Solids\ (g/g)}$$

Where:

$A - Spectroquant\ Reading\ Concentration$
$V - Volume\ of\ Dilution\ (L)$
$M - Mass\ of\ Sludge\ used\ in\ sample\ preperation\ (g)$

Standard Operation Procedure – Total Kjeldahl Nitrogen

1. Scope and Application

- This Kjeldahl method determines nitrogen in the tri-negative state. This fails to account for nitrogen in the form of ozide, azine, azo, hydrazone, nitrate, nitrite, nitro.
- **Kjeldahl nitrogen is the sum of organic nitrogen and ammonia nitrogen.** Organic nitrogen: includes proteins, peptides, nucleic acids and urea.
- Typical organic nitrogen concentrations vary from a few hundred mg/L in some lakes to more than 20mg/L in raw sewage.
- This macro- Kjeldahl method is applicable for samples containing either low or high concentrations of organic nitrogen but requires but requires a relatively large sample volume for low concentrations.

2. Summary

- In the presence of sulphuric acid, potassium sulfate, and cupric sulphate catalyst, amino nitrogen of many organic materials is converted to ammonium.
- Free ammonia is also converted to ammonium.
- After addition of base, the ammonia is distilled from an alkaline medium and absorbed in boric or sulphuric acid.
- The ammonia may be determined colorimetrically, by ammonia selective electrode or by titration with a standard mineral acid.
- The titrimetric and selective electrode methods of measuring ammonia in the distillate are suitable for determining a wide range of organic nitrogen concentrations.

4. Sampling

- Nitrate: During Kjeldahl digestion, nitrate in excess of 10mg/L can oxidize a portion of the ammonia released from the digested organic nitrogen, producing N_2O, resulting in a negative interference.
- Inorganic salts and solids: The acid and salt content of the Kjeldahl digestion reagent is intended to produce a digestion temperature of about 380ºC.
- If the sample contains a very large quantity of salts or inorganic solids the temperature may rise to 400ºC during digestion at which point pyrolytic loss of nitrogen occurs. To prevent this increase in temperature add more sulphuric acid to maintain an acid-salt balance.

3. Interferences

- The most reliable results are obtained on fresh samples.
- If an immediate analysis is not possible, preserve samples for Kjeldahl digestion by acidifying to pH 1.5 to 2.0 with concentrated sulphuric acid and storing at 4ºC.
- Do not use $HgCl_2$ because it will interfere with ammonia removal.

5. Safety Precautions

- Handle concentrated sulphuric acid with care.
- Always use safety goggles, gloves and laboratory coat while working in laboratory
- After the analysis clean bottles and beakers with clear water keep it for drying
- Dispose the used gloves after completion of analysis
- Clean the hands using antiseptic soap
- Disinfect hands after washing with soap
- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.

6. Apparatus

- Velp Digestion apparatus: DK 20 slim. F30100181, S/N 214130 .
- SMS Scrubber F307C0199
- JP recirculating water pump F30620198
- Kjeldahl flasks with a total capacity of 800ml yield the best results. Digest over a heating device adjusted so that 250ml water at an initial temperature of 25ºC can be heated to a rolling boil in about 5 min. The temperature range should be 375 to 385ºC for effective digestion.
- Distillation apparatus: UDK 127 Distilling Unit F30200183 s/N 126145
- 300ml TKN flasks.

7. Sampling Preparation – Fecal Sludge

- Weigh out between 1.8g and 2g of well mixed fecal sludge sample.
- Place the weighed out sample into a blender with 250ml of distilled water.
- Blend for 30 seconds.
- Transfer the blended mixture into a volumetric flask and top up to 1L with distilled water.
- Transfer the 1L solution to a plastic bottle and store in the cold room.

8. Reagents

Digestion Reagent:

Kjeldahl tablets or powder: Free of Hg, Se.

3.5g K2SO4 and 0.5g CuSO4

Boric Acid – 4 %

Dissolve 40g of Boric acid into 1L of distilled water.

Concentrated Sulphuric acid

Sulphuric acid – 0.1N

Dissolve 2.8ml of concentrated sulphuric acid into 1L of distilled water

Sodium Hydroxide – 35%

Dissolve 350g of NaOH into 1L of distilled water

Mixed Indicator

Mix methyl red (20mg) and bromocresol green indicator (100mg) top up to 100ml ethanol. Make up every month.

Standard

A solution of 30mg/N is prepared by weighing 0.1607g glycine dissolving in distilled water and diluting to 1L in a volumetric flask.

Waste water / Sludge	20-100ml (70ml)	15-20 TKN
Final effluent	140ml	
Outfalls	100ml	4-5 TKN

9. Procedure

Sample Preparation

- Weigh out between 1.8g and 2g of well mixed fecal sludge sample.
- Place the weighed out sample into a blender with 250ml of distilled water.
- Blend for 30 seconds.

- Transfer the blended mixture into a volumetric flask and top up to 1L with distilled water.
- Transfer the 1L solution to a plastic bottle and store in the cold room.

Heating Block

- Place 50ml of mixed diluted sample into 300ml Kjeldahl flask for raw or primary sewage, wastewater or 140ml for ponds, rivers or final effluents.
- Add a glass rod to the tube, and 5 boiling stones.
- Add slowly 10ml of concentrated sulphuric acid, 1kjeltab (or 2 spatulas powder), swirl to dissolve- wait approx 15min or overnight if sample as a high organic/fat content and then place onto digestion unit.
- **Add 1000ml of 32% NaOH into reagent bottle 2, screw in bottle(clockwise) and push the bubbling tube to the bottom.**
- **Temperature range is set, follow program 1**: 380 ºC for 60min,

- **Place suction cap onto tubes and open tap until a steady flow of water is reached (2L/min)**.
- **Set pump to Mode A, air flow No: 4 until temperature of heating block reaches 200** °C.
- **Then set pump to Mode B: air flow No: 4 until end of digestion.**
- **Reactivate Mode B -100 % of the maximum air flow – if SO_3 gas emission is too much.**
- Boil briskly at 380°C until dense fumes of SO_3 are evolved and a pale green color is obtained.
- The required temperature i.e. 380°C is usually reached after an hour.
- Keep pump running for 30min after samples are fully digested and heating block is switched off.
- If sample is too little before fully digested, add 10ml concentrated sulphuric acid and remember to increase the vol of sodium hydroxide used during the distillation.
- Digestion takes about 3 hrs – colour changes from blue to dark green to black to colorless/pale green.
- Switch off the heating block, pump and the water supply.
- Replace water in the water bath and replace the NaOH in reagent bottle 2.

- Wait for samples to cool then distill samples.

Distillation

- Prepare absorption solution by placing 25ml of 4% boric acid in a 250ml conical flask and insert under the condenser outlet with the tip below the surface of boric acid.
- Lower collected distillate free from contact with the condenser tip and continue distillation for 1 or 2 minutes to cleanse the condenser.
- Enter distillation programme as follows:

Vol of water	Add 50ml to tube manually
Phenolphthalein indicator	10 drops to tube manually
Vol of NaOH	50ml (if 10ml sulphuric acid used in digestion)
	200ml (if 30ml sulphuric acid used in digestion**)**
Distillation time	3min

Sample in tube turns purple with addition of NaOH - above pH 11 before distillation

Distillate in flask should reach around pH 8 before titrating.

Titration

Titrate distillate against 0.1N sulphuric acid with mixed methyl red (0.02g) bromocresol green indicator (0.1g) top up to 100ml ethanol.

Colour change: from blue to pale pink

Calculation

$$Nitrogen\ (mg/L) = \frac{(Titration - Blank)(0.1)(14)(1000)}{Sample\ Volume\ (ml)}$$

0.1 - Concentration of sulphuric acid used in titration

14 - Atomic weight of Nitrogen

1000 - Conversion of g to mg

$$Nitrogen\ in\ Wet\ Sample\ (mg/g) = \frac{(Titration - Blank)(0.1)(14)(1000)}{Sample\ Volume\ (ml)} \times \frac{V}{M}$$

$$Nitrogen\ in\ Wet\ Sample\ (g/g) = \frac{Nitrogen\ in\ Wet\ Sample\ (mg/g)}{1000}$$

$$Nitrogen\ in\ Dry\ Sample\ (g/g) = \frac{Nitrogen\ in\ Wet\ Sample\ (g/g)}{Total\ Solids\ (g/g)}$$

Where:

V = Volume of dilution (L)

M = mass of sludge used in sample preparation (g)

10. Precision and Accuracy

mg TKN/L %SD %Error

APPROVAL OF STANDARD OPERATING PROCEDURE

PRG Head: Prof Buckley Signature: ……

Date: ……

Author Merlien Reddy: Signature: ……

Date: ……..

Standard Operation Procedure – Ammonium analysis (Cat. No. 1.00683)

1. Scope and Field of Application

Measuring range 2.6 – 193 mg/L NH_4^+

2. Principle

Ammonium nitrogen (NH_4 –N) occurs partly in the form of ammonium ions and partly as ammonia. A pH-dependent equilibrium exists between the two forms. In strongly alkaline solution ammonium nitrogen is present almost entirely as ammonia, which reacts with a chlorinating agent to form monochloramine. This in turn reacts with thymol to form a blue indophenol derivative that is determined photometrically.

3. Interferences

4. Sampling

- Preferably collect samples in glass bottles.

5. Safety Precautions

- Handle concentrated acid with cares
- Always use safety goggles, gloves and laboratory coat while working in laboratory
- After the analysis clean bottles and beakers with clear water keep it for drying
- Dispose the used gloves after completion of analysis

- Clean the hands using antiseptic soap
- Disinfect hands after washing with soap
- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.

6. Apparatus

- Spectrophotometer

7. Reagents

- NH_4-1 – Non-Hazardous
- NH_4-2 – Sodium nitroprusside (≥ 10% - <20%); Troclosene sodium, dehydrate (≥ 1% - <2.5%)
- NH_4-3 – Non-Hazardous

8. Calibration

9. Sample Preparation

Fecal samples are diluted by blending 1.8g -2g sample into 1L of distilled water, as described in detail below:

- Weigh out 1.8g – 2g fecal sample using an analytical balance and add to a blender with 100mL distilled water and blend.
- Add blended sample to a 1L volumetric flask and dilute to 1L using distilled water.
- Swirl flask until sample is completely dissolved.

Filtration

Filter paper dimensions: diameter = 47mm, pore size = 0.45 microns

- Filter the diluted solution using a Buchner funnel.
- Collect the filtrate for analysis.

10. Procedure

- Pipette 5.0ml of pretreated sample (20-30°C) into a test tube.
- Add 0.6ml of reagent NH_4-1(20-30°C) with a pipette and mix.
- Add 1 level blue micro-spoon (in the cap of the NH_4-2 reagent bottle).
- Add Shake vigorously until the reagent is completely dissolved.
- Leave to stand for 5 min (reaction time A)
- Add 4 drops of Reagent NH_4-3 and mix.
- Leave to stand for 5 min (reaction time B) then fill the sample into the cell and measure in the photometer.

11. Disposal of Waste Chemicals

- Collect waste in a labeled 2.5L bottle for collection from Waste Tech.

12. Calculations

$$Wet\ Sample\ Concentration\ (g/g)\ = \frac{A}{1000} \times \frac{V}{M}$$

$$Dry\ Sample\ Concentration\ (g/g)\ = \frac{Wet\ Sample\ Conc.\ (g/g)}{Total\ Solids\ (g/g)}$$

Where:

$A - Spectroquant\ Reading\ Concentration$
$V - Volume\ of\ Dilution\ (L)$
$M - Mass\ of\ Sludge\ used\ in\ sample\ preperation\ (g)$

Standard Operation Procedure – Nitrates Analysis (Cat. No. 1.09713)

1. Scope and Field of Application

Measuring range 0.4 – 110.7 mg/L NO_3^-

2. Principle

In sulphuric and phosphoric solution nitrate ions react with 2,6-dimethylphenol(DMP) to form 4-nitro-2,6-dimethylphenol that is determined photometrically.

3. Interferences

- Check the chloride content with Meckoquant Chloride Test. Samples containing more than 1000mg/l Cl^- must be diluted with distilled water.
- Check the nitrite content, if necessary, eliminate interfering nitrite ions(stated amounts apply for nitrate contents of up to 50 mg/l)
- To 10ml of sample add approx. 50mg of amidosulphuric acid and dissolve.
- The pH of this solution must be within the range of 1-3. Adjust, if necessary with sulphuric acid.
- Filter turbid samples

4. Sampling

- Preferably collect samples in glass bottles.

5. Safety Precautions

- Handle concentrated acid with cares
- Always use safety goggles, gloves and laboratory coat while working in laboratory
- After the analysis clean bottles and beakers with clear water keep it for drying
- Dispose the used gloves after completion of analysis
- Clean the hands using antiseptic soap
- Disinfect hands after washing with soap
- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.

6. Apparatus

- Heating Block for nitrate measurement
- Spectrophotometer

7. Reagents

- NO3-1 – Sulphuric acid (≥25% - <50%); Phosphoric acid (≥25% - <50%)
- NO3-2 – 2-Propanol (≥15% - <20%); Xylenol (≥0.25% - <1%)

8. Calibration

To check the photometric measurement system (test reagents, measurement device, handling) and the mode of working, the nitrate standard solutions CRM, 0.500mg/l NO3-N, CAT No. 125036, 2.50mg/l NO3-N Cat No 125037 and Combicheck 20 can be used. Combicheck 20 also contains an addition solution for determining sample-dependent interference (matrix effects).

9. Sample Preparation

Fecal samples are diluted by blending 1.8g -2g sample into 1L of distilled water, as described in detail below:

- Weigh out 1.8g – 2g fecal sample using an analytical balance and add to a blender with 100mL distilled water and blend.
- Add blended sample to a 1L volumetric flask and dilute to 1L using distilled water.
- Swirl flask until sample is completely dissolved.

Filtration

Filter paper dimensions: diameter = 47mm, pore size = 0.45 microns

- Filter the diluted solution using a Buchner funnel.
- Collect the filtrate for analysis.

10. Procedure

Note: Procedures according to Merck operational Manual for test kits (Nitrate Test 1.09713.0001)

- Analyze immediately after sampling.
- Pipette 4.0ml of reagent NO3-1 into a dry test tube.
- Add with pipette 0.50ml of pretreated sample (5-25°C), do not mix.
- Add with pipette 0.5ml of reagent NO3-2.(wear eye protection, the mixture becomes hot) and mix, holding only the upper part of the tube.
- Leave the hot reaction to stand for 10min (reaction time).

- Do not cool with water.
- Fill the sample into the rectangular cell and measure in the photometer.

11. Disposal of Waste Chemicals

- Dilute 10 ml into 1000ml.
- Slowly add $NaCO_3$ until pH 6-8 is reached.
- Flush down the sink with excess water.

12. Calculations

$$Wet\ Sample\ Concentration\ (g/g)\ = \frac{A}{1000} \times \frac{V}{M}$$

$$Dry\ Sample\ Concentration\ (g/g)\ = \frac{Wet\ Sample\ Conc.\ (g/g)}{Total\ Solids\ (g/g)}$$

Where:

$A - Spectroquant\ Reading\ Concentration$
$V - Volume\ of\ Dilution\ (L)$
$M - Mass\ of\ Sludge\ used\ in\ sample\ preperation\ (g)$

Standard Operation Procedure – Nitrites analysis (Cat. No. 1.14547)

1. Scope and Field of Application

Measuring range 0.03 - 2.30 mg/L NO_2^-

2. Principle

In acidic solution nitrite ions react with sulfanilic acid to form a diazonium salt, which in turn reacts with N-(1-naphthyl) ethylenediamine dihydrochloride to form a red-violet azo dye. This dye is determined photometrically.

3. Interferences

4. Sampling

- Preferably collect samples in glass bottles.

5. Safety Precautions

- Handle concentrated acid with cares
- Always use safety goggles, gloves and laboratory coat while working in laboratory
- After the analysis clean bottles and beakers with clear water keep it for drying
- Dispose the used gloves after completion of analysis
- Clean the hands using antiseptic soap
- Disinfect hands after washing with soap

- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.

6. Apparatus

- Spectrophotometer

7. Reagents

- NO_2-1 – Sulphanilic acid >50%; N-(1-Naphthyl)ethylenediamine dihydrochloride <10%

8. Calibration

To check the photometric measurement system (test reagents, measurement device, handling) and the mode of working, the nitrite standard solutions CRM, 0.200mg/l NO2-N, CAT No. 125041 can be used. Sample-dependent interference (matrix effects) can be determined by means of standard addition.

9. Sample Preparation

Fecal samples are diluted by blending 1.8g -2g sample into 1L of distilled water, as described in detail below:

- Weigh out 1.8g – 2g fecal sample using an analytical balance and add to a blender with 100mL distilled water and blend.
- Add blended sample to a 1L volumetric flask and dilute to 1L using distilled water.
- Swirl flask until sample is completely dissolved.

Filtration

Filter paper dimensions: diameter = 47mm, pore size = 0.45 microns

- Filter the diluted solution using a Buchner funnel.
- Collect the filtrate for analysis.

10. Procedure

- Pipette 5.0ml of pretreated sample (5-25°C) into a test tube.
- Add 1 level blue micro-spoon (in the cap of the NO_2-1 reagent bottle).
- Shake vigorously until the reagent is completely dissolved.
- The pH must be within the range 2.0 -2.5.
- Adjust the pH, if necessary with sodium hydroxide solution or sulphuric acid.
- Leave to stand for 10 min (reaction time) then fill the sample into the cell and measure in the photometer.

11. Disposal of Waste Chemicals

- Dilute 10 ml into 1000ml.
- Flush down the sink with excess water.

12. Calculations

$$Wet\ Sample\ Concentration\ (g/g)\ = \frac{A}{1000} \times \frac{V}{M}$$

$$Dry\ Sample\ Concentration\ (g/g)\ = \frac{Wet\ Sample\ Conc.\ (g/g)}{Total\ Solids\ (g/g)}$$

Where:

$A - Spectroquant\ Reading\ Concentration$
$V - Volume\ of\ Dilution\ (L)$
$M - Mass\ of\ Sludge\ used\ in\ sample\ preperation\ (g)$

Standard Operation Procedure – Phosphate and total P Analysis (Cat. No. 1.14848); (Cat. No. 1.14543)

1. Scope and Field of Application

The measurement of total phosphorus and phosphate is essential for performance studies on the struvite reactor. The phosphate concentration in influent and effluent gives indication on the performance of the reactor operation whereas the total P values (influent and effluent) demonstrate the effectiveness of the filtration material used. The recovery can be calculated based on these measurements.

(Phosphate) Measuring range 0.02 – 11.46 mg/L P_2O_5

(Total Phosphate) Measuring range 0.11 – 11.46 mg/L P_2O_5

2. Principle

In sulfuric solution orthophosphate ions react with molybdate ions to form molybdophosphoric acid. Ascorbic acid reduces this to phosphomolybdenum blue (PMB) that is determined photometrically.

3. Interferences

- Sample for phosphate analysis must be pretreated by filtration (0.45µm) to remove most of turbidity (interferes with photometric measurement)

- In case of total P sample mustn´t be filtrated! The filtration step would remove already precipitated struvite during urine storage and thus false the analysis
- In any case urine should be diluted at least 1:100 to avoid matrix effects
- (Other interferences are mentioned in operational manual of test kits)

4. Sampling

- Preferably collect samples in glass bottles.

5. Safety Precautions

- Handle concentrated acid with cares
- Always use safety goggles, gloves and laboratory coat while working in laboratory
- After the analysis clean bottles and beakers with clear water keep it for drying
- Dispose the used gloves after completion of analysis
- Clean the hands using antiseptic soap
- Disinfect hands after washing with soap
- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.

6. Apparatus

- Heating Block for Total P measurement
- Spectrophotometer

- Glass ware: Use acid washed glassware for determining low concentrations of orthophosphates. Phosphate contamination is common because of its absorption on glass surfaces. Avoid using commercial detergents containing phosphate. Clean all glassware with hot dilute HCL and rinse well with distilled water. Preferably reserve the glassware only for phosphate determination and after use, wash and keep filled with water until needed. If this is done, acid treatment is required only occasionally.

7. Reagents

Phosphate Test

- PO_4-1 – Sulphuric Acid (≥25% - <50%)
- PO_4-2 – Non-Hazardous

Total Phosphate Test

- P-1K – Sodium nitrate (≥50% - ≤100%)
- P-2K – Sulphuric Acid (≥10% - <15%)
- P-3K – Non-Hazardous

8. Calibration

To check the photometric measurement system (test reagents, measurement device, handling) and the mode of working, Spectroquant® CombiCheck 10 can be used. Besides a standard solution with 0.80 mg/l PO4-P, the CombiCheck 10 also contains an addition solution for determining sample-dependent interferences (matrix effects).

9. Sample Preparation

Fecal samples are diluted by blending 1.8g -2g sample into 1L of distilled water, as described in detail below:

- Weigh out 1.8g – 2g faecal sample using an analytical balance and add to a blender with 100mL distilled water and blend.
- Add blended sample to a 1L volumetric flask and dilute to 1L using distilled water.
- Swirl flask until sample is completely dissolved.

Filtration

Filter paper dimensions: diameter = 47mm, pore size = 0.45 microns

- Filter the diluted solution using a Buchner funnel.
- Collect the filtrate for analysis.

10. Procedure

Note: Procedures according to Merck operational Manual for test kits (Phosphate 1.14848.0001 and total P 1.14543.0001)

Ortho-Phosphate measurement:

- Pipette 5.0 ml pretreated (diluted and filtered) sample into a test tube.
- Reagent PO4-1 5 drops Add and mix.
- Reagent PO4-2 1 level blue microspoon, add and shake vigorously until the reagent s completely dissolved
- Leave to stand for 5 min (reaction time), then fill the sample into the cell, and measure in the photometer.

Total P measurement:

- Digestion for the determination of total phosphorus (Wear eye protection!):
    - Pipette 5.0 ml pretreated sample into a reaction cell
    - Add 1 dose Reagent P-1K, close the cell tightly, and mix.
    - Heat the cell at 120 °C in the preheated thermoreactor for 30 min.
    - Allow the closed cell to cool to room temperature in a test-tube rack.
    - Do not cool with cold water!
- shake the tightly closed cell vigorously after cooling.
- Add 1 dose reagent P-2K, close the cell tightly, and mix.
- Add 1 dose reagent P-3K, close the cell tightly, and shake vigorously until the reagent is completely dissolved.
- Leave to stand for 5 min (reaction time), then measure the sample in the photometer.

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Sample Analysis

Note: Procedure according to Merck operational Manual for test kits (Phosphate 1.14848.0001 and total P 1.14543.0001)

Ortho-Phosphate measurement:

- Pipette 8.0 mL distilled water into a test tube.
- Add 0.5 mL pretreated sample with a micro-pipette and mix.
- Add 0.5 mL Reagent PO4-1 with a micro-pipette and mix.
- Add 1 dose Reagent PO4-2 and shake vigorously until the reagent is completely dissolved.
- Leave to stand for 5min (reaction time), and then fill the sample into the cell (10-mm cuvette) and measure in the photometer.

Total P measurement:

- Digestion for the determination of total phosphorus (Wear eye protection!):
  - Pipette 5.0 mL pretreated sample into a reaction cell.
  - Add 1 dose Reagent P-1K, close cell tightly, and mix.
  - Heat the cell at 120℃ in the preheated thermoreactor for 30 min.
  - Allow the closed cell to cool to room temperature in a test-tube rack.
  - Do not cool with cold water!

- Shake the tightly closed cell vigorously after cooling.
- Add 1 dose Reagent P-2K, close the cell tightly, and mix.
- Add 1 dose Reagent P-3K, close the cell tightly, and shake vigorously until the reagent is completely dissolved.
- Leave to stand for 5 min (reaction time), then measure the sample in the photometer.

Procedure (Using Standard Solution - Reagent R-1)

Note: The error caused by the photometric measurement system and the mode of operation can be determined by means of the standard solution. This is used **without dilution** in place of the sample solution.

Basic Procedure: Proceed according to the instructions given in the package insert of the respective test kit and in the manual of the photometer used (as described in the total P measurement procedure using UD samples). In this case, however, use **undiluted reagent R-1** in place of the sample without adjusting the pH!

Detailed Procedure:

Total P measurement using a standard solution (reagent R-1):

- Digestion for the determination of total phosphorus (Wear eye protection!):
  - Pipette 5.0 mL **undiluted reagent R-1** into a reaction cell.
  - Add 1 dose Reagent P-1K, close cell tightly, and mix.
  - Heat the cell at 120℃ in the preheated thermoreactor for 30 min.
  - Allow the closed cell to cool to room temperature in a test-tube rack.
  - Do not cool with cold water!

- Shake the tightly closed cell vigorously after cooling.
- Add 1 dose Reagent P-2K, close the cell tightly, and mix.
- Add 1 dose Reagent P-3K, close the cell tightly, and shake vigorously until the reagent is completely dissolved.
- Leave to stand for 5 min (reaction time), then measure the **standard sample** in the photometer.

11. Disposal of Waste Chemicals

- Dilute 10 ml into 1000ml.
- Slowly add NaCO3 until ph 6-8 is reached.
- Flush down the sink with excess water.

12. Calculations

$$Wet\ Sample\ Concentration\ (g/g)\ = \frac{A}{1000} \times \frac{V}{M}$$

$$Dry\ Sample\ Concentration\ (g/g)\ = \frac{Wet\ Sample\ Conc.\ (g/g)}{Total\ Solids\ (g/g)}$$

Where:

$A - Spectroquant\ Reading\ Concentration$
$V - Volume\ of\ Dilution\ (L)$
$M - Mass\ of\ Sludge\ used\ in\ sample\ preperation\ (g)$

Standard Operation Procedure –Solids

Introduction

Solids refer to matter suspended or dissolved in water or wastewater. Solids may affect water or effluent quality adversely in a number of ways. Solids analyses are important in the control of biological and physical wastewater treatment processes and for assessing compliance with regulatory agency wastewater effluent limitations.

Total Solids is the term applied to material residue left in the vessel after evaporation of a sample and its subsequent drying in an oven at a defined temperature. Total solids includes total suspended solids, the potion of solids retained by a filter and total dissolved solids, the portion that passes through the filter of 2.0um or smaller. Fixed Solids, is the term applied to residue of total, suspended or dissolved solids after heating to dryness for a specified time at a specified temperature. The weight loss on ignition is called volatile solids.

Total Solids Dried at 103-105ºC

1. Scope and Field of Application

Total Solids are determined in a wide variety of liquid and semi-liquid materials. These include portable waters, domestic and industrial waters, polluted waters and sludge produced from treatment processes. It is of particular importance for the efficient operation of a treatment plant.

2. Principle

A known volume of well-mixed sample is evaporated to dryness in a porcelain crucible in a hot air oven at 105ºC, the solids remaining are cooled and weighed. The residual material in the crucible is classified as total solids, and may consist of organic, inorganic, dissolved, suspended or volatile matter.

3. Interferences

- Highly mineralized water with a significant concentration of calcium, magnesium, chloride and sulphate may be hygroscopic and require prolonged drying, proper desiccation and rapid weighing.
- Exclude large, floating particles from the sample if it is determined that their inclusion is not desired in the final result.
- Disperse visible floating oil and grease with a blender before withdrawing sample portion for analysis. Because excessive residue in the dish may form a water-trapping crust.

4. Sampling

- Mix the sample well to suspend solids uniformly.
- Remove the test portion rapidly before any settling of solid matter occurs.
- Use a measuring cylinder and not a pipette for sludge and wastewater samples.
- Use a crucible for feces.
- Use a volume or mass of sample to ensure a measurable residue- limit sample to no more than 200mg residue
- Suitable aliquots: Liquid samples – 100ml, Sludges -30ml, feces 10-20g.

5. Safety Precautions

- Always use safety goggles, gloves and laboratory coat while working in laboratory
- Wear gloves suitable for withstanding high temperatures when removing crucibles from the oven.
- After the analysis clean bottles and beakers with clear water keep it for drying

- Dispose the used gloves after completion of analysis
- Clean the hands using antiseptic soap
- Disinfect hands after washing with soap
- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.

6. Apparatus

- 50ml capacity evaporating porcelain crucibles
- Desiccator
- Drying oven
- Four – place Analytical Balance

7. Reagents

- Nil.

8. Calibration

- Check the temperature throughout the oven area by placing a calibrated thermometer on each shelf, after 30mins, check temperature at each level against oven setting.
- Adjust oven setting if necessary.
- If temperatures are uneven on the shelves, check insulation.

9. Procedure

Prepare Crucible

- If volatile solids are to be measured ignite clean crucible at 550ºC for 1hr in the furnace. If only total solids are to be measured, heat clean crucible to 103-105ºC for 1h. Store and cool dish in a desiccator until needed. Weigh immediately before use**……W1g**

Sample Analysis

- Measure out appropriate volume (30ml) /mass (10-20g) that will yield a residue between 2.5 and 200mg of a well mixed sample using correct volume measuring cylinder or analytical balance**…..Vml…Wg**. Transfer quantitatively to the weighed crucible, rinsing the cylinder with small volumes of distilled water to dislodge heavy particles. Add washings to the crucible.
- Place in hot oven at 103-105°C for 24hrs
- ***Dry sample for at least 1hr in an oven 103-105°C, to dish in desiccator to balance temperature and weigh. Repeat cycle of drying, cooling , desiccating and weighing until a constant weight is obtained, or until weight change is less than 4% of previous weight or 0.5mg, whichever is less.***
- Remove the next day and cool for 15 minutes and weigh**…..W2g**

10. Calculation

$$Total\ Solids\ in\ Sample\ (mg/l) = \frac{(W_2 - W_1)g \times 100\ 000}{V_{sample}\ (ml)}$$

$$Total\ Solids\ in\ Wet\ Sample\ (g/g) = \frac{(W_2 - W_1)g}{W_{sample}\ (g)}$$

$$Moisture\ Content\ (g) = W_{sample}(g) - [(W_2 - W_1)]g$$

Total Suspended Solids Dried at 103-105ºC

1. Scope and Field of Application

Suspended solids are useful determinants in the analysis of polluted, re-use and waste waters. It is used to evaluate the strength of domestic/industrial waste waters and to determine the efficiency of treatment units, such as settling tanks, biological filters, and the activated sludge. Use of glass fiber filter pads is preferred to crucibles because of the saving in filtration time and the only prior preparation necessary is drying in an oven for 30mins at 105ºC.

2. Principle

A measured volume of well shaken is vacuum filtered through a dried pre-weighed 110mm diameter glass fiber filter. The filters and residue is dried to a constant weight at 103-105ºC. The increase in weight of the filter represents the total suspended solids.

3. Interferences

- Exclude isolated large floating particles.
- Samples high in dissolved solids must be washed adequately.
- Loss in mass of the rinsed glass fiber filters must be taken into the final calculation.
- The larger the sample, the smaller the factor applied in the calculation, but avoid prolonged filtrations.

4. Sampling

- Take the sample at a point of turbulence to ensure that it is truly representative.
- Mix sample thoroughly and remove test portion rapidly before segregation occurs.
- Use appropriate volume measuring cylinder and not pipettes.

5. Safety Precautions

- Exercise care when using glassware, vacuum pumps and ovens.
- Good housekeeping and cleanliness are essential for obtaining accurate results.

6. Apparatus

- Four- place Analytical balance
- 110mm diameter funnel and flask
- Vacuum pump

7. Reagents

- Nil

8. Calibration

- The analytical balance are checked and serviced weekly.

9. Sample Preparation – Fecal Sludge

- Weigh out between 1.8g and 2g of well mixed fecal sludge sample.
- Place the weighed out sample into a blender with 250ml of distilled water.
- Blend for 30 seconds.
- Transfer the blended mixture into a volumetric flask and top up to 1L with distilled water.
- Transfer the 1L solution to a plastic bottle and store in the cold room.

10. Procedure

Dry Filter Paper

- Use 110mm glass fiber filter paper Whatman No 4(20-25um)
- Mark the filter paper with a pen
- Place papers on the stainless steel mess of appropriate size
- Position on top shelf in oven at 105ºC for 30min .
- If volatile solids are to be measured ignite at 550 ºC for 15min in a furnace.
- Transfer to desiccator
- Cool for 20 minutes before weighing

Weigh Filter Paper

- Transfer filter paper rapidly to balance
- Note mass**(W1)**grams, to fourth decimal place

Prepare for Analysis

- Place filter pare into a 110mm diameter funnel, with the marking on the lower side
- Measure out appropriate volume to yield between 2.5 and 200mg dried residue of well mixed sample

- Place funnel into flask with side arm attached to a vacuum pump.
- Apply pump
- Wet paper with distilled water to seal edges of the paper to surface of the funnel
- Pour sample onto the filter paper, keeping sample in the middle of the paper.
- When filtration is complete. Remove paper by placing the end of a small thin spatula along the edge of the filter paper and lift slowly.
- Remove the paper with a pair of tweezers, taking care not to tear the paper.
- Fold paper twice to form a triangle enclosing sample residue. This seals the residue in the filter paper and protects it from contact with air.

Dry and Weigh

- Place triangles on a stainless steel mess
- Place in oven at 105ºC for 2hrs
- Remove from oven and place in desiccator
- Cool to room temperature
- Weigh after 20 mins, as rapidly as possible
- Note mass **(W2)**grams

11. Calculation

$$Total\ Suspended\ Solids\ (g/ml) = \frac{(W_2 - W_1)}{V_{sample}(ml)}$$

$$Total\ Suspended\ Solids\ in\ Wet\ Sample\ (g/g) = TSS\ (g/ml)\ \times DF$$

$$Total\ Suspended\ Solids\ in\ Dry\ Sample\ (g/g) = \frac{TSS_{wet\ sample}}{Total\ Solids\ (g/g)}$$

W_1 = weight of filter paper before oven (105ºC) (g)

W_2 = weight of residue + filter paper after oven(105ºC) (g)

DF = Dilution Factor

Fixed and Volatile Solids Ignited at 550ºC

1. Principle

The residue from the above methods is ignited to constant weight at 550ºC. The remaining solids represent the fixed total, dissolved or suspended solids while the weight lost on ignition is the volatile solids. The determination is useful in control of wastewater treatment plant operation because it offers a rough estimate of the amount of organic matter present in the solid fraction of wastewater, activated sludge and industrial wastes.

2. Interferences

- Negative errors in the volatile solids may be produced by loss of volatile matter during the drying.

3. Apparatus

- Muffle Furnace
- As above

4. Procedure

- Ignite residue from the total solids to constant weight in a muffle furnace at a temperature of 550ºC.
- Have furnace up to temperature before inserting sample.
- Usually 2 hr for VIP and sludge samples,15-20min for waste water(200mg residue)
- Let the crucible cool partially in air until most of the heat has dissipated
- Transfer to a desiccator for final cooling. Do no overload the desiccator
- Weigh dish as soon as it has cooled to balance temperature.

5. Calculation

$$Volatile\ Solids\ in\ Wet\ Sample\ (g/g) = \frac{(B - C)}{W_{sample}(g)}$$

$$Volatile\ Solids\ in\ Dry\ Sample\ (g/g) = \frac{VS_{wet\ sample}}{Total\ Solids(g/g)}$$

$$Fixed\ Solids\ in\ Wet\ Sample(g/g) = \frac{(C - D)}{W_{sample}(g)}$$

$$Fixed\ Solids\ in\ Dry\ Sample(g/g) = \frac{FS_{wet\ sample}}{Total\ Solids(g/g)}$$

B = weight of residue + crucible before ignition - 550ºC (g)

C = weight of residue + crucible after ignition -550ºC (g)

D = weight of crucible (g)

6. Precision and Accuracy

mg Total Solids/L %SD %Error

APPROVAL OF STANDARD OPERATING PROCEDURE

PRG Head: Prof Buckley Signature: ……

Date: ……

Author Merlien Reddy: Signature: ……

Date: ……..

Standard Operation Procedure – Calorimetric Tests

1. Scope and Application

Heats of combustion as determined in an oxygen bomb calorimeter are measured by a substation procedure in which the heat obtained from the sample is compared with the heat obtained from combustion of a similar amount of benzoic acid or other standardizing material whose calorific value is known. These measurements are obtained by burning a representative sample in a high pressure oxygen atmosphere within a bomb. The energy released by this combustion is absorbed within the calorimeter and the resulting temperature change within the absorbing medium is noted. The heat combustion of the sample is then calculated by multiplying the temperature rise in the calorimeter by the previously determined energy equivalent or heat capacity determined from a standardizing material.

2. Summary

Calorimetry is the science of measuring quantities of heat, as distinct from temperature. The instruments used for such measurements are known as calorimeters. The oxygen bomb calorimeters, which are the standard instruments for measuring calorific values of liquid and solid combustible samples.

The calorific value (heat of combustion) of a sample may be broadly defined as the number of heat units liberated by a unit mass of a sample when burned with oxygen in an enclosure of constant volume.

3. Sampling

- Dry sample for 24 hours @ 105 ° C in a crucible.
- Grind dried sample to a powder form.
- Weigh out a gram of sample into the sample vial.

- The bomb should never be charged with a sample which will release more than 8000 calories when burned in oxygen, and the initial oxygen pressure should never exceed 40 atmospheres (590psi).
- Never charge the bomb with more than 1g of combustible material.

4. Safety Precautions

- Do not use to much sample. The standard bomb 1108 cannot be expected to withstand the effects of combustible charges which liberate more than 8000 calories frequently. This generally limits the total weight of combustible material (sample plus combustion aid) to not more than 1.1gram.
- Do not charge the bomb with more oxygen that is necessary to obtain complete combustion. It is best to use the lowest gas pressure that will give complete combustion. Lower gas pressure permit higher gas temperatures and greater turbulence, both of which help to secure better combustion. The range is 20-35 atmospheres.
- Keep all parts of the bomb-especially the o-rings, insulated electrode assemblies and valves- in good repair at all times.
- Do not fire the bomb if gas bubbles are leaking from the bomb when it is submerged in water.
- Stand back from the calorimeter for at least 15 seconds after firing and keep clear of the top of the calorimeter. If the bomb should explode, it is likely that the force of the explosion will be directly upward.

5. Apparatus

- Parr 6200 Oxygen Bomb Calorimeter
- 1180P Oxygen Combustion Bomb

6. Reagents

- Benzoic acid tablets for standardization.

7. Calibration

8. Procedure

1. Open oxygen gas cylinder, flow rate is already set to (400kPa), do not alter.
2. Check that distilled water chamber is filled to the mark.
3. Turn on the calorimeter and activate the pump and heater. Allow at least 20 minutes for the calorimeter to warm up and the jacket temperature to stabilize at 29 °C.
4. The calorimeter is ready to begin testing. The START key will be available at this time.
5. Fill the calorimeter bucket with 2L of distilled water. Set the bucket in the calorimeter.
6. SAMPLE PREP:
   - 1 g 24hr @105 °C dried blended sample into capsule. Tie cotton tread which is used as a fuse to ignite the sample onto heat wire in the bomb.
   - When contact is made through the heating wire, the tread will ignite, drop into the sample cup and ignite the sample.
7. Care must be taken when moving the bomb head from the support stand to the bomb cylinder.
8. Check the sealing ring to be sure it is in good condition and moisten it with a bit of water so it will slide freely into the cylinder and push it as far down as it can go.
9. Close the bomb and pressurize with oxygen. The pressure connection to the bomb is made with a slip connector on the oxygen hose.
10. Slide the connector onto the inlet valve body and push it as far as it can go.

11. Press the O2 FILL button and Step back until bomb is filled. Takes 1min.

12. Remove the gas connection and attach the lifting handle to the two holes in the side of the screw cap and lower the bomb into the water partially. **Press the banana plugs onto the two ignition wires firmly into the terminal sockets on the bomb head before the head is completely immersed in water.**

13. Note: If bubbles continue to rise from the bomb after the air in the screw cap has escaped the test must be stopped. Do not fire the bomb until the leak has been corrected.

14. Close the calorimeter cover. This lowers the stirrer and thermistor probe into the bucket. Make sure that the bucket thermistor does not touch the bucket or the bomb when the lid is lowered.

15. Select **determination under OPERATING MODE**.

16. After pressing the START key, the calorimeter will prompt the operator for BOMB ID number, sample ID number, sample weight and spike weight.

17. The calorimeter will now take over and conduct the test.

18. During the time it is establishing the initial equilibrium, the status bar will display PREPERIOD.

19. Just before it fires the bomb, at about 12min, it will sound a series of short beeps to warn the user to move away from the calorimeter.

20. Once the bomb has been fired the status bar will display POSTPERIOD.

21. Read of calorific value from screen.

22. Remove the bomb from the chamber after 3 minutes and depressurize bomb by opening the valve knob slowly. After all the pressure has been released, unscrew the cap and lift the head straight out to avoid sticking.

23. Remove the chamber containing the ash

24. Wipe the inside of the bomb clean and proceed with next sample.

Standard Operating Procedure – Penetrometer Use for Testing of Faecal Sludge

1. Scope and field of application

Faecal sludge samples are obtained from different sources like: (i) mixed excreta samples from community ablution blocks and (ii) mixed excreta samples from household urine diversion toilets.

Characterization of the rheology (flow properties) of samples is necessary for the accurate design of new toilet systems.

Human excreta can potentially host significant levels of pathogens, and as such is classified as a biologically hazardous material. Suitable precautions must be taken when handling samples.

2. Principle

Semi-Automatic Cone Penetrometer is used to carry out penetration tests that will be used to calculate shear stress. Penetration tests are performed on products (e.g petroleum, civil engineering) industries to determine consistency and shear stability of lubricating greases or soil for design and quality control purposes.

The test is based on the measurement of penetration into the soil (/sludge sample) of a standardized cone of specified mass.

3. Safety Precautions

General

The following general safety precautions should be taken:

- Cover any small open wounds with waterproof dressings – if large open wounds then do not carry out laboratory work.
- Always use gloves, laboratory coat and closed shoes while working in the laboratory.
- Wear a face-shield when disposing of samples down the sluice (risk of splash back).
- Dispose of samples as specified by the Faeces Sample Disposal SOP.
- Clean all soiled equipment thoroughly after use.
- Any equipment that will be taken out of the laboratory into a 'clean' environment (e.g. camera) should be handled only with clean gloves and disinfected using 70% ethanol spray after use.
- Dispose of the used gloves in the appropriate waste bin after sample handling and disposal and cleaning of equipment is complete.
- Clean hands using antiseptic soap.
- Disinfect hands after washing with soap.

Where mixed samples are being handled (i.e. those from field location sources such as community ablution blocks), additional care must be taken as sharps may be present in the faecal matter. Samples should not be handled directly with gloved hands, but rather with a spoon or spatula.

Maintain 'clean' and 'dirty' work areas

The basement laboratory where excreta samples are processed should be considered in its entirety a 'dirty' area, however within this 'clean zones' should be designated for any items that will later be taken out of the laboratory:

- Sample boxes and equipment used to handle samples should only be placed on wipe-clean surfaces - plastic or metal top workbenches or trays.

- Any 'clean' items that will be taken out of the laboratory – e.g. camera and paper forms used to record results – should be kept on a clean tray or segregated clean area of the workbench.
- 'Clean' items should only be handled whilst wearing clean gloves.

Specific safety precautions for the penetrometer:

- Use metal trays as the work area for loading the penetrometer cup with the sample and for the placement of tools which are in direct contact with the sample, in order to prevent any environmental contamination from the sample.
- Use paper sheets and **DO NOT** place samples directly on any surface.
- Clean and disinfect the equipment after use.

Protective safety equipment must be worn at all time in the lab. Special dust masks that prevent smell are essential and must be worn when working with samples.

4. Apparatus

- Cone penetrometer with standard cone of mass 50 grams.
- Sample cup of diameter 55 mm and 40 mm deep.
- A stopclock or stopwatch readable to 1 s.
- Two palette knives or spatulas.

5. Materials & Equipment Required

- Metal trays (preferably non-stick)
- Paper sheets for cleaningfaeces, equipment and general cleaning
- Laboratory spoons for loading the penetrometer cup with the sample
- Rubber spatula to scrape any sample out of a cup
- A knife or flat sharp object to scrape excess material over the cup.
- Brushes for washing instruments after use
- 70% ethanol for disinfection of equipment, splashes and spills

6. Procedure for Measurement

1. Take a sample of about 300g (to fill the cup) from the container.

2. Push a portion of the mixed sludge into the cup with a palette knife taking care not to trap air. Strike off excess soil with the straightedge to give a smooth level surface.

3. With the penetration cone locked in the raised position lower the supporting assembly so that the tip of the cone just touches the surface of the soil. When the cone is in the correct position a slight movement of the cup will just mark the soil surface. Lower the stem of the dial gauge to contact the cone shaft and record the reading of the dial gauge to the nearest 0.1 mm.

4. Release the cone for a period of 5 ± 1 s. If the apparatus is not fitted with an automatic release and locking device take care not to jerk the apparatus during this operation. After locking the cone in position lower the stem of the dial gauge to contact the cone shaft and record the reading of the dial gauge to the nearest 0.1 mm. Record the difference between the beginning and end of the drop as the cone penetration.
5. Lift out the cone and clean it carefully to avoid scratching.

6. Repeat 1 to 5.

7. If the difference between the first and second penetration readings is not more than 0.5 mm record the average of the two penetrations.

8. If the second penetration is more than 0.5 mm and less than 1 mm different from the first, carry out a third test. If the overall range is then not more than 1 mm record the average of the three penetrations. If the overall range is more than 1 mm remove the soil from the cup, remix and repeat 1 to 5 until consistent results are obtained.

Notes:

- Results for fluids with a low viscosity tested at low shear rates, may be inaccurate due to the effects of surface tension (e.g. water)
- Samples can dehydrate over time. It is recommended that the cup is covered for long tests (over an hour in duration)
- All tests will be performed at a standard temperature of 25°C

At the end:

- Dispose of all waste samples according the SOP for faeces sample disposal.
- Clean all apparatus after use.
- Store remaining samples appropriately.
- Update sample database with tests carried out on each sample.

7. Calibration

Standard Operating Procedure – Rheometer Use for Testing of Faecal Matter

1. Scope and field of application

Excreta samples are obtained from several sources including (i) segregated faeces samples from individual donors, (ii) mixed excreta samples from community ablution blocks and (iii) mixed excreta samples from household urine diversion toilets.

Characterization of the rheology (flow properties) of samples is necessary for the accurate design of waterless toilet systems.

Human excreta can potentially host significant levels of pathogens, and as such is classified as a biologically hazardous material. Suitable precautions must be taken when handling samples.

2. Principle

An Anton Parr MCR51 rheometer is used to carry out a number of rheological tests on feces samples. These include: flow curves, amplitude and frequency sweeps, variable temperature tests and stress recovery tests. A number of different measuring systems exist (cone-cup, plate-plate, building materials cell) – each is suited to a different type of sample.

3. Safety Precautions

General

The following general safety precautions should be taken:

- Cover any small open wounds with waterproof dressings – if large open wounds then do not carry out laboratory work.
- Always use gloves, laboratory coat and closed shoes while working in the laboratory.
- Wear a face-shield when disposing of samples down the sluice (risk of splash-back).
- Dispose of samples as specified by the Faeces Sample Disposal SOP.
- Clean all soiled equipment thoroughly after use.
- Any equipment that will be taken out of the laboratory into a 'clean' environment (e.g. camera) should be handled only with clean gloves and disinfected using 70% ethanol spray after use.
- Dispose of the used gloves in the appropriate waste bin after sample handling and disposal and cleaning of equipment is complete.
- Clean hands using antiseptic soap.
- Disinfect hands after washing with soap.

Where mixed samples are being handled (i.e. those from field location sources such as community ablution blocks), additional care must be taken as sharps may be present in the faecal matter. Samples should not be handled directly with gloved hands, but rather with a spoon or spatula.

Maintain 'clean' and 'dirty' work areas

The basement laboratory where excreta samples are processed should be considered in its entirety a 'dirty' area, however within this 'clean zones' should be designated for any items that will later be taken out of the laboratory:

- Sample boxes and equipment used to handle samples should only be placed on wipe-clean surfaces - plastic or metal top workbenches or trays.
- Any 'clean' items that will be taken out of the laboratory – e.g. camera and paper forms used to record results – should be kept on a clean tray or segregated clean area of the workbench.
- 'Clean' items should only be handled whilst wearing clean gloves.

Specific safety precautions for the rheometer:

- Use metal trays as the work area for loading the rheometer cup with the sample and for the placement of tools which are in direct contact with the sample, in order to prevent any environmental contamination from the sample.
- Clean and disinfect all equipment after use.

4. Apparatus

- Anton Parr MCR51 Rheometer
- 27 mm cone-cup attachment
- 32 mm cone-cup attachment
- Plate-plate attachment
- Building materials cell

5. Materials & Equipment Required

- Metal trays (preferably non-stick)
- Paper sheets for cleaning faeces, equipment and general cleaning

- Laboratory spoons for loading the rheometer cup with the sample
- Rubber spatula to scrape any sample out of a cup
- Brushes for washing instruments after use
- 70% ethanol for disinfection of equipment, splashes and spills

6. Procedure for Measurement

For a cone-cup setup, the following procedure applies:

- Ensure all equipment and apparatus is clean and clear of obstructions
- Run the Anton PaarRheometer Software
- Initialise the Rheometer using the software
- Install the desired cup attachment
- Insert the corresponding cone measuring instrument and allow the rheometer software to load the calibrated data for that instrument
- Select the desired test worksheet/template, from the list of available worksheets within the software
- Set the temperature of the rheometer to a desired temperature, and allow the temperature calibration program to complete
- Raise the measuring device and load the sample when prompted to the specified volume marker in the cup
- Lower the measuring instrument and allow the sample to 'relax' for 30 minutes, to negate the effects of structural deformation
- Initialise the selected experiment and wait for the experimental run to complete
- Detach the measuring device from the machine head and raise the head

- Unload the sample from the cup, and clean the cup and measuring instrument immediately
- To perform another run, repeat all steps from selecting a worksheet

Notes:

- A larger cone-cup diameter will produce more accurate results, since there is a larger surface area
- Results for fluids with a low viscosity tested at low shear rates, may be inaccurate due to the effects of surface tension (e.g. water)
- Samples can dehydrate over time. It is recommended that the cup is covered for long tests (over an hour in duration)
- All tests will be performed at a standard temperature of 25°C
- Stool samples may have significantly different moisture concentrations on either end of the sample, in which case the sample must be separated and the two ends treated as different samples.
- Run each sample once, before it has to be reloaded.
- 24.5ml of sample is required for a run with the CC27 setup (65ml for CC39XL)

For a plate-plate setup, the following procedure applies:

- Ensure all equipment and apparatus is cleaned and cleared of obstructions
- Run the Anton PaarRheometer Software
- Initialise the Rheometer and
- Insert the corresponding plate measuring instrument and allow the rheometric software to load the calibrated data for that instrument
- Perform and allow the 'Set zero gap' programme to complete for the first use of the attached instrument
- Select the desired test worksheet, from the list of available worksheets

- Set the temperature of the rheometer to a desired temperature, and allow the temperature calibration program to complete
- Load the sample in the centre of the base plate when prompted
- Move the instrument into the measuring position and trim the excess sample
- Initialise the selected experiment and wait for the experimental run to complete
- Clean the plate and measuring instrument immediately
- To perform another run, repeat all steps from selecting a worksheet

Dispose of all waste samples according the SOP for faeces sample disposal.

Clean all apparatus after use.

Store the remaining samples appropriately.

Update sample database with tests carried out on each sample.

Updating the sample database

- Data should be transferred from paper records to the electronic sample database as soon as possible.
- The updated database should be saved in the following location:

\\Un\.DBNPRG1_VOL1.ENG.und\PRG\PRG-RESEARCH\PROJECTS\Gates Reinvent the Toilet Challenge RN95\8 Project areas\Sample analysis and database

- The database filename should be edited to reflect the date it was saved on – e.g. a version saved on the 1 June 2012 would be saved with a filename starting with 20120601.

7. Calibration

- Each instrument used in the rheometer must have a 'motor calibration' and 'inertia calibration' service performed at least every 90 days.

Standard Operation Procedure – Sludge Volume Index for Faecal Sludge

1. Scope and Field of Application

Sludge Volume Index (SVI) is an indication of the sludge settleability in the final clarifier. It is a useful test that indicates changes in the sludge settling characteristics and quality.

By definition, the SVI is the volume of settled sludge in millilitres occupied by 1 gram of dry sludge solids after 30 minutes of settling in a 1000 ml graduated cylinder or a settleometer.

2. Safety Precautions

- Always use safety goggles, gloves and laboratory coat while working in laboratory
- Wear gloves suitable for withstanding high temperatures when removing crucibles from the oven.
- After the analysis clean bottles and beakers with clear water keep it for drying
- Dispose the used gloves after completion of analysis
- Clean the hands using antiseptic soap
- Disinfect hands after washing with soap
- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.

3. Apparatus

- 1L graduated cylinder
- Stop watch
- Four – place Analytical Balance
- 50ml capacity evaporating porcelain crucibles
- Desiccator

4. Procedure

Determine the suspended solids concentration of a well-mixed sample of the suspension (do the suspended solids test, see **Total solids SOP**)

Determine the 30 min settled sludge volume as follows:

Preparing a mixed liquor

- Measure out sludge
- Place sludge in volumetric flask and top up to 1L
- Mix well
- Place solution in graduated cylinder until the 1L marking
- Allow it to settle for 30 minutes
- After the time period, read the marking to determine the volume occupied by the settled sludge and the reading is expressed in terms of mL/L and this is figure is known as the SV value

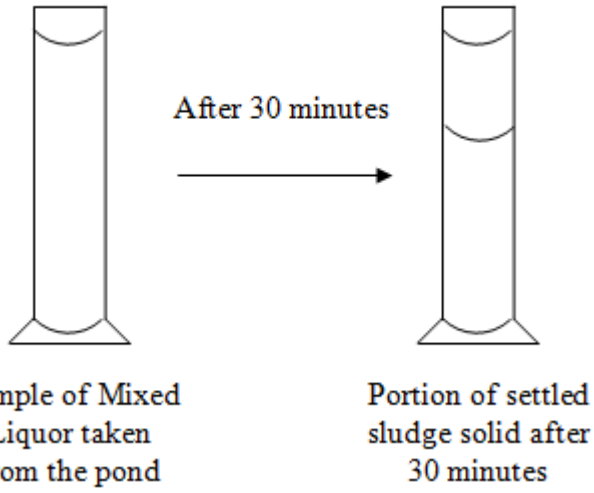


Figure 1 Experimental set-up

5. Calculation

Calculate the settled sludge volume as follows:

$$SVI\ (mg/ml) = \frac{settled\ sludge\ volume\ (ml/L) \cdot 1000}{suspended\ solids\ (mg/L)}$$

6. References

1. http://www.epa.ie/downloads/advice/water/wastewater/epa_water_%20treatment_manual_primary_secondary_tertiary1.pdf
2. http://www.wastewatersystem.net/2010/10/correlation-between-sludge-volume-index.html
3. http://www.norweco.com/html/lab/test_methods/2710cfp.htm

Standard Operation Procedure – Thermal Conductivity Testing

1. Scope and Application

- This document provides instructions on testing minimal volumes of powder, liquids, feces,pit latrine(VIP) samples using TCi small volume test kit(SVTK).
- Testing with the C-Therm TCi can directly measure thermal conductivity and thermal effusivity.
- It can indirectly measure diffusivity, heat capacity, the R-value or depth of penetration.

2. Summary

The SVTK was developed for testing minimal volumes of liquid volumes of fluid material. Reducing the volume of sample material required for an effective thermal conductivity measurement is extremely important in the testing of energetic materials whereby larger samples pose a significant safety concern. The use of the accessory has also been applied widely in the testing of various materials that are doped with extremely expensive filters(gold, diamonds etc.) that are in limited supply.

3. Interferences

- If any cell is red , the measurement is not valid. Repeat the measurement.
- Check the R^2 value for each measurement. If the R^2 value is less than 0.995, the measurement is not valid.
- An orange cell means that the thermal conductivity or thermal effusivity value is outside of the calibration range of that material group.
- To enter density:

- Click on material, add, density value,save.

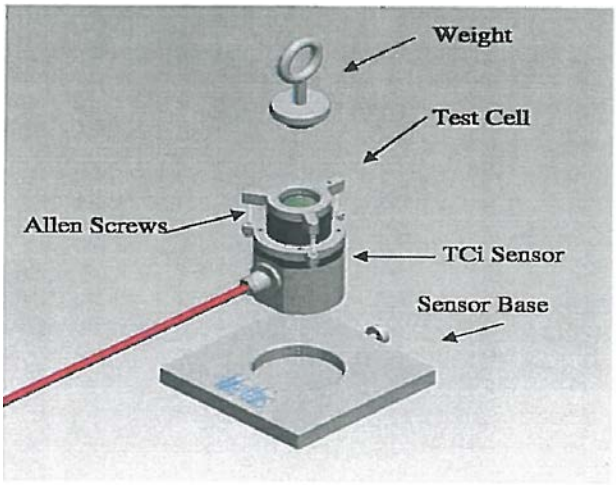
4. Sampling

5. Safety Precautions

6. Apparatus

SVTK P/N:

- Weight
- Test Cell, Allen screws,
- TCi sensor
- Sensor Base
- Measuring spoons(1/8 and ¼)



7. Reagents

8. Calibration

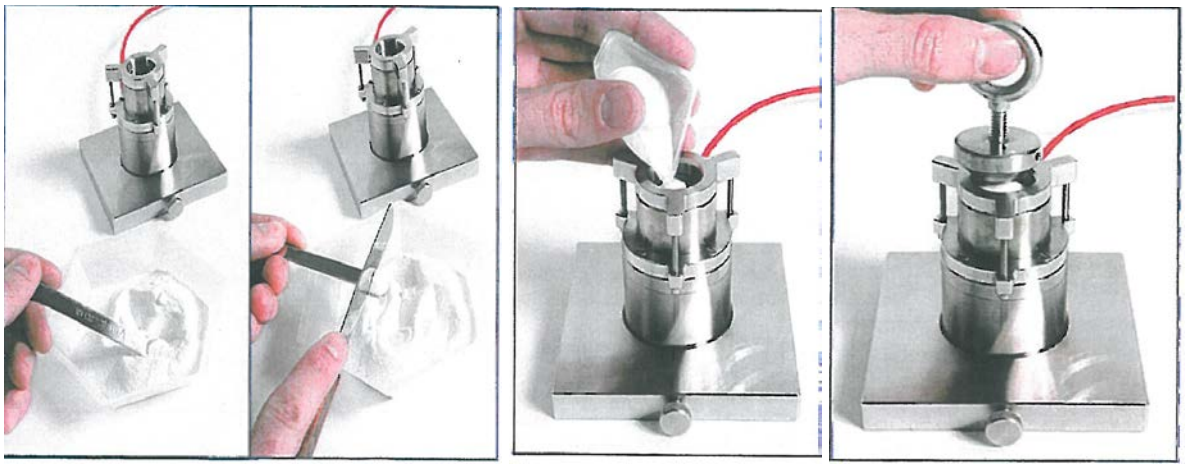
9. Procedure

DENSITY:

SOLID TESTING:

- Fill the 1/8 teaspoon (0.63ml) with the sample to be investigated.
- Level off the excess sample by scraping off the excess with a spatula by making a horizontal movement.
- Care must be taken to prevent compaction of the sample in the teaspoon (e.g. Vibrations, rearranging sample with spatula, and tapping on the teaspoon).
- The sample remaining in the teaspoon is the specimen.
- Transfer the sample to a weighing dish.
- Repeat the above steps 3 times for a total volume of specimen of approximately 3/8 teaspoon or 1.8ml.
- Place the weight onto the sample so that it seats on the rim of the test cell.NB:If the sample to be measured weighs more than 150g,omit the weight.
- Monitor the sensor temperature via the TCi software until it is stable and the sensor , sample and environment have all reached a state of thermal equilibrium.

- Initiate the test sequence within the TCi software.



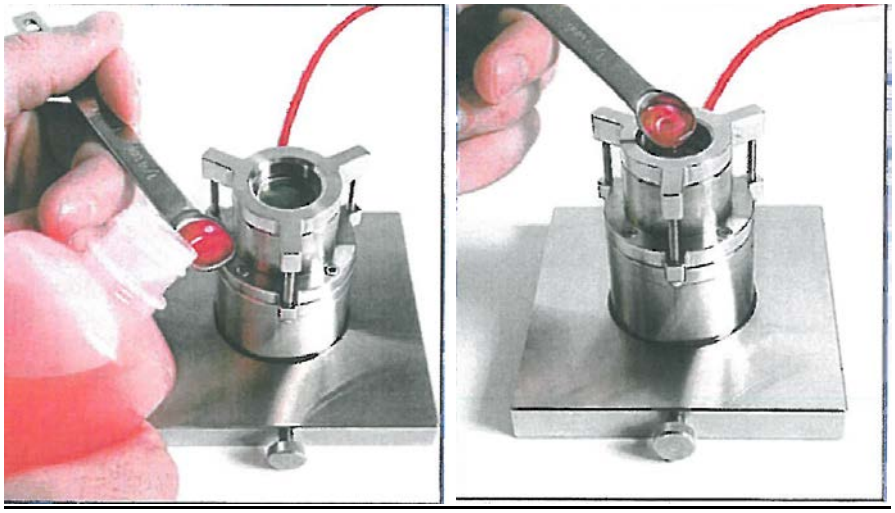
CLEANING:

- Pour out the contents of the sample from the test cell or remove it with a paper towel.
- Place sensor upside down and remove the test cell by gradually unfastening the three screws I a sequenced manner. Use a 3/32 Allen wrench.
- Remove the sensor test and clean with either soap and water, water or propyl alcohol.
- To test again place the test cell on the sensor and place upside down in order to have easy access to the screws.
- Tighten gradually and in sequence until the test cell seats perfectly flat against the sensor housing surface.

LIQUID TESTING:

- Measure 1.25ml(1/4 tsp) of total liquid volume of specimen**.**
- Transfer this volume directly to the test cell.
- Place the quick clamp cap on the test cell.

- Use of the cap is optional but will prevent any undesirable evaporation of the liquid from the cell.
- Monitor the sensor temperature via the TCi software until it is stable and the sensor, sample and environment have all reached a state of equilibrium.
- Initiate the test sequence within the TCi software.



TCi Quick Card

For technical support call 1-506-462-7204 or email support@ctherm.com

Material Type	Minimum Thickness	Sample Preparation	Contact Agent
Liquids	1 mm	Fill 50 mL beaker to 35 mL mark. Place sensor in beaker.	None
Powders	1 mm	Fill 50 mL beaker to 30 mL mark. Place sensor in beaker.	
Foams	2 mm	Place sample on sensor. Place weight on sample.	
Polymers	5 mm	Place contact agent on sensor. Place sample on sensor. Place weight on sample.	**-20°C to 5°C**: 3 drops of **glycol** **5°C to 70°C**: 3 drops of **water** **70°C to 200°C**: **Wakefield 120 Thermal Joint Compound**
Ceramics	5 mm		
Metals	5-12 mm		

Testing: General Procedure

Step 1 Prepare the materials to be tested. Inspect surface for dirt/damage.

Step 2 Position the material on the sensor. Place weight if applicable.

Step 3 Click the New Test button on the toolbar.

Step 4 Select the project.

Step 5 Click the Next button.

Step 6 Select the test method to be used.

Step 7 Select the material group and material to be used. If no suitable record exists, click the New button, enter the name of the group or material, and click the Save button.

Step 8 Click the Next button.

Step 9 Select the instrument.

Step 10 Select the sensors.

Step 11 Select the contact agent.

Step 12 Click the Start Test button.

Testing Tips

- Wear gloves while handling samples to avoid thermal contamination.
- When using a thermal chamber, allow samples and sensor to equilibrate to temperature for 2 hours prior to testing and for 10 minutes every time the door is opened.

Importing Files

Step 1 Select the type of record to import from the Tools menu.

Step 2 Select the file to import.

Step 3 All records contained in the file are displayed.

Step 4 Click the Import button.

Exporting Files

Test results, user calibration results, calibration methods, and test methods can be exported and imported. All records with the exception of notes are exported and imported with the test results, user calibration results, calibration methods, or test methods.

Step 1 Select the type of file to export from the Tools menu.

Step 2 Enter keywords in the parameter fields.

Step 3 Click the Search button or press the Enter key.

Step 4 Select the records to export from the displayed list.

Step 5 Click the Next button.

Step 6 Select a destination for the export file.

Step 7 Click the Export button.

Creating a Test Method

Step 1 Open the test method table.

Step 2 Click the Add button.

Step 3 Enter a name for the test method.

Step 4 Select a project.

Step 5 Select a calibration method.

Step 6 Enter the delay before the first measurement (optional).

Step 7 Enter the minimum measurement period (optional).

Step 8 Enter the number of measurements to be taken. If zero is entered, measurements will be taken until the test is stopped by the user.

Step 9 Enter the number of sensors to be used. If zero is entered, any number of sensors can be selected when beginning a test.

Step 10 Enter the number of samples per sensor per measurement. This is the number of times the sensor fires during a single measurement interval.

Step 11 Select the prompts to be displayed.

Step 12 Click the Save button.

Changing Units

Step 1 Select Change Units of Measure from the Tools menu.

Step 2 Select the units.

Step 3 Click the OK button.

Step 4 Logout and restart the software.

Reference Material Tests

Calibration Material Group	Reference Material
Liquids and Powders	Distilled Water
Foams	LAF 6720*
Polymers	Pyrex
Ceramics	Pyroceram
Metals	Phosphor Bronze*

Step 1 Prepare the reference material and sensor.

Step 2 Click Reference Material Test from the Tools menu.

Step 3 Click the Next button.

Step 4 Select an Instrument.

Step 5 Select the calibration method.

Step 6 Select the sensor(s) to be used.

Step 7 Click the Next button.

Step 8 Select the reference material bin (Foam and Metal)

Step 9 Confirm the ambient temperature.

Step 10 Click the Update button (if temperature was incorrect).

Step 11 Click the Get Sample button. All results should be within 5% of the displayed predicted value.

Step 12 Click the Finish button.

C-THERM TECHNOLOGIES

Standard Operation Procedure – Density of Faecal Sludge

Introduction

Density is the relationship between the mass (m) and volume (V) of a substance.

Bulk density (Db) is a measure of mass per unit volume. It is used as a measure of wetness, volumetric water content, and porosity. Factors that influence the measurement include; organic matter content, the porosity, and material structure. The reference mass of the material is taken after oven drying.

Particle density or solid density (Dp) represents only the weight of dry material per unit volume of the material solids; the pore space is not included in the volume measurement.

The Porosity (PS) of a material is pore space portion of the material volume occupied by air and water.

1. Scope and Field of Application

Bulk density is determined by oven-drying a known volume of sample and the mass of the dry sample measured. Solid or particle density is determined by one of two methods:

1. The displacement technique or
2. The saturation method.

Once the bulk and particle density values are known, it is a straightforward calculation to determine pore space

.

2. Safety Precautions

- Always use safety goggles, gloves and laboratory coat while working in laboratory
- Wear gloves suitable for withstanding high temperatures when removing crucibles from the oven.
- After the analysis clean bottles and beakers with clear water keep it for drying
- Dispose the used gloves after completion of analysis
- Clean the hands using antiseptic soap
- Disinfect hands after washing with soap
- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.

3. Apparatus

- 50ml capacity evaporating porcelain crucibles
- Desiccator
- Drying oven
- Four-place Balance
- 100ml measuring cylinder
- Sample holding tube
- 7.5ml measuring spoon

4. Reagents

- Nil.

5. Calibration

- Check the temperature throughout the oven area by placing a calibrated thermometer on each shelf, after 30mins, check temperature at each level against oven setting.
- Adjust oven setting if necessary.
- If temperatures are uneven on the shelves, check insulation.

6. Procedure

Prepare Crucible

- Heat a porcelain crucible in an oven for 2 hrs at 103-105ºC. Cool for 15 minutes in a desiccator and weigh…….W1g

Sample Analysis

- Measure out appropriate volume of 7.5ml sample. Transfer into the crucible and weigh (crucible+sample).....W2g
- Place in hot oven at 103-105ºC overnight.
- Remove the next day and cool for 15 minutes and weigh…..W3g

- Suspend a sample holding tube into into a water in the 100ml measuring cylinder and record the volume of water.....V1ml
- Carefully transfer the dry sample into a holding tube, suspend the tube with sample in the water and record the volume.....V2ml (displacement technique).

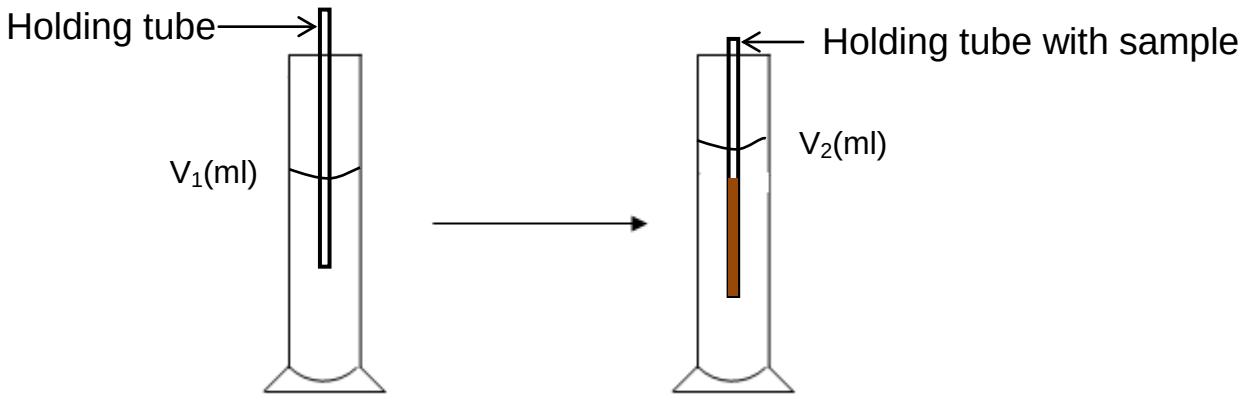


Figure 1 Experimental set-up (displacement technique)

Calculation

$$Db_{wet}(g/ml) = \frac{W2 - W1}{V_t}$$

Where:

W2-W1 = Wet mass of sample
V_t = Total volume of sample (7.5ml)

$$Db_{dry}(g/ml) = \frac{W_s}{V_t}$$

Where:

W_s = Oven dry mass of the sample = W3-W1 (g)
V_t = Total volume of the sample, pore volume + solid volume (7.5ml).

Particle Density, Dp

Particle density values represents only the weight of dry sample per unit volume of the sample solids; the pore space is not included in the volume measurement.

$$Dp(g/ml) = \frac{W_s}{V_s}$$

Where:
W_s = Oven dry mass of the sample (g)
V_s = Volume of the solids (ONLY) = V_2-V_1(ml).

$$PS = \frac{1 - Db_{dry}}{Dp}$$

Standard Operation Procedure – Liquid Limit

1. Scope and Application

The liquid limit is the moisture content at which a soil passes from the liquid phase into the plastic phase determined experimentally. It is used to classify a soil, particularly when the plastic limit is also known and hence the plasticity index can be established. This method is adapted from the British Standard BS 1377-2:1990 for use on pour flush sludge.

2. Summary

The cone penetrometer provides a static test depending on soil shear strength to determine its liquid limit. The cone is dropped from a height into the sample and the penetration is recorded and correlated to the moisture content of the sample. The moisture content is increased continuously and the test repeated, until an approximate linear graph can be produced. The water content corresponding to a penetration of 20mm is the liquid limit of the sample in question.

3. Interferences

Coarse particles can affect the reproducibility of the test and should be removed from the test sample whenever possible.

4. Sampling

Store samples in plastic or glass containers, taking note of the date the sample was taken.

5. Safety Precautions

- Always use safety glasses, gloves, closed shoes and laboratory coat when working in the laboratory
- Dispose of sample in the sluice when test completed
- Thoroughly clean all equipment after use
- Any equipment that will be taken out of the laboratory should be handled with clean gloves only and disinfected with 70% ethanol after use
- Dispose of used gloves when analysis completed
- Wash hands with antiseptic soap and disinfect with 70% ethanol when analysis completed
- Use metal trays to place soiled equipment when not in use
- Avoid spillage and contact with skin. In latter case use copious washings with cold water and call for medical attention

6. Apparatus

- Flat glass plate, 10mm thick and 500mm square
- Two palette knives or spatulas
- One or more metal cups (55±2mm in diameter and 40±2mm deep)
- Cone Penetrometer
- Evaporating dish of approx. 150mm diameter

- Apparatus for moisture content determination
- A wash bottle or beaker filled with distilled water
- A corrosion resistant airtight container
- A metal straightedge approx.100m long or a straight-bladed spatula
- A stopwatch/stopclock readable to 1s

7. Sample Preparation –Fecal Sludge

- Do not allow sample to dry before testing.
- Sample should be of a soil in its natural state, or where the material remaining on a 425 µm test sieve has been removed from the soil.

8. Reagents

- Nil

9. Calibration

10. Procedure

1. Place 300g of prepared sample on the glass plate.
2. Mix sample with the two palette knives for approx. 10 mins adding distilled water if necessary to achieve first cone penetration reading of 15mm.
3. Push the mixed sample into a metal cup with the palette knife and strike of the excess creating a smooth, level surface using the straightedge.
4. Place the metal cup on the base of the apparatus, ensuring the penetration cone is in locked a raised position.

5. Lower the penetration cone carefully until it just touches the surface of the sample, the correct position is indicated if the cone just scratches the surface when the cup is moved.
6. When the cup has been placed in the correct position, lower the stem of the dial gauge until it just touches the cone shaft.
7. Set the dial gauge to zero (to the nearest 0.1mm)
8. Release the cone for 5s ±1s. Lock the cone into position after the 5s have lapsed and lower the stem of the dial gauge again to touch the cone shaft. Read the dial gauge to the nearest 0.1mm, this value is recorded as the cone penetration.
9. Lift the cone from the cup and clean it carefully.
10. Place some more wet sample into the cup, ensuring no air is trapped and repeat steps 3 through 9.
11. If the difference between the first and second penetrations is less than 0.5mm, record the average value. If the difference is greater that 0.5mm but less than 1mm, repeat the test a third time and if the overall range is no greater than 1mm record the average of the three values. If the overall range is greater than 1mm remove the sample from the cup and repeat procedure from step 2.
12. Take the moisture content of approximately 10g of sample where the cone penetrated the cup.
13. Repeat procedure from step 2 at least 3 times using the same sample, to which increments of distilled water has been added.
14. Go from drier to wetter samples, until a cone penetration range of approximately 15mm to 25mm has been reached over the course of at least 4 test runs and values are evenly distributed.
15. Wash and dry the cup each time the sample of soil is removed to facilitate the addition of water.
16. If the soil is left in the open for extended periods of time, cover with evaporating dish or damp cloth to avoid drying.
17. Plot moisture content against cone penetration to obtain a linear graph from a straight line that best fits the plotted points.
18. The moisture content corresponding to the cone penetration of 20mm is reported as the liquid limit, to the nearest whole number.

Standard Operation Procedure – Plastic Limit

1. Scope and Application

The plastic limit of a soil is the experimentally determined moisture content at which it is too dry to behave plastically. It is used in conjunction with the liquid limit to produce the Plasticity Index to classify soils. This method is adapted from the British Standard BS 1377-2:1990 for use on pour flush sludge.

2. Summary

The sample is moulded into a thin thread of approximately 3mm and from a ball until cracks appear in the thread, longitudinally and transversely. The moisture content at which the cracks appear is the plastic limit.

3. Interferences

Results are subject to the interpretation of the researcher hence variations in results may occur. When this method is applied to soils heat from the hands is expected to dry out the soil to lead to the transverse and longitudinal shearing. The length of time taken to dry out the sludge may be extended due to the necessity of wearing latex gloves

4. Sampling

Store samples in plastic or glass containers, taking note of the date the sample was taken.

5. Safety Precautions

- Always use safety glasses, gloves, closed shoes and laboratory coat when working in the laboratory
- Ensure there are no holes in gloves when excessive handling of sludge is required
- Dispose of used gloves when analysis completed
- Dispose of sample in the sluice when test completed
- Thoroughly clean all equipment after use
- Any equipment that will be taken out of the laboratory should be handled with clean gloves only and disinfected with 70% ethanol after use
- Wash hands with antiseptic soap and disinfect with 70% ethanol when analysis completed
- Use metal trays to place soiled equipment when not in use
- Avoid spillage and contact with skin. In latter case use copious washings with cold water and call for medical attention

6. Apparatus

- Flat glass plate, to mix and roll samples (10mm thick, 300mm square)
- 2 palette knives or spatulas
- Apparatus to determine moisture content

- A length of rod 3mm in diameter and 100mm long

7. Sample Preparation –Fecal Sludge

- Do not allow sample to dry before testing.
- Sample should be of a soil in its natural state, or where the material remaining on a 425 µm test sieve has been removed from the soil.

8. Reagents

- Nil

9. Calibration

10. Procedure

1. Approximately 20g of sample is placed on glass plate for mixing
2. Allow sample to dry until it is plastic enough to be shaped into a ball
3. Mould the sample into a ball between the fingers and then roll it between the palms until the heat of the hands has made it dry enough that small cracks appear on the surface.
4. Divide sample into 2 subsamples of approximately 10g, carrying out a separate determination for each subsample.
5. Divide each subsample into 4 more approximately equal samples and apply the following steps to each sample
6. Mould the sample between the fingers to equally distribute the moisture and then roll the sample into a thread of approximately 6mm between the thumb and first finger.
7. Roll the thread on the glass plate with the fingers, from their tip to the second knuckle using enough pressure to reduce the diameter to approx. 3mm in 5 to 10 forward and backward rolls.
8. It is important to maintain a constant rolling pressure.

9. Pick up the sample and mould between fingers, reproduce a thread shape and repeat steps 7 and 8.
10. Continue step 9 until the thread shears both longitudinally and traversely when it is rolled to 3mm diameter, which is determined using the rod. Do not collect the pieces and reproduce the thread, as the first crumbling point is the plastic limit.
11. Place the pieces of the thread in a container and seal with a lid. Place the pieces of all four threads in the one container and determine the moisture content.
12. Repeat steps 5 through 11 for the second set of 4 samples.
13. If the moisture content of the 2 samples differs by more then 0.5% the whole test must be repeated.
14. Calculate the average of the two moisture content values and round to the nearest whole number. This is the plastic limit.

Standard Operating Procedure – Density Measurement of Faecal Matter

1. Scope and field of application

The following method for density measurement is sufficient to provide an approximate value for the density of a sample of faecal matter, but cannot be used for accurate measurements. Density values of faecal matter are of relevance in the design of toilet systems and emptying equipment for on-site sanitation facilities.

Faeces can potentially host many transmittable diseases and pathogens, and as such is classified as a biologically hazardous material. Suitable precautions must be taken when handling samples.

2. Principle

The mass of a known volume of feaces is measured and volume determined by direct calculation.

3. Interferences

Inaccuracies may arise from the following sources:

- The measuring spoon is not completely filled with sample
- The sample is compressed in the process of filling the spoon
- Sample is not cut off completely flush with the surface of the measuring spoon

Due to these potential sources of error, this method is only intended to provide an approximate value for the density of the sample.

4. Safety Precautions

General

The following general safety precautions should be taken:

- Cover any small open wounds with waterproof dressings – if large open wounds then do not carry out laboratory work.
- Always use gloves, laboratory coat and closed shoes while working in the laboratory.
- Wear a face-shield when disposing of samples down the sluice (risk of splashback).
- Dispose of samples as specified by the Faeces Sample Disposal SOP.
- Clean all soiled equipment thoroughly after use.
- Any equipment that will be taken out of the laboratory into a 'clean' environment (e.g. camera) should be handled only with clean gloves and disinfected using 70% ethanol spray after use.
- Dispose of the used gloves in the appropriate waste bin after sample handling and disposal and cleaning of equipment is complete.
- Clean hands using antiseptic soap.
- Disinfect hands after washing with soap.

Where mixed samples are being handled (i.e. those from field location sources such as community ablution blocks), additional care must be taken as sharps may be present in the faecal matter. Samples should not be handled directly with gloved hands, but rather with a spoon or spatula.

Maintain 'clean' and 'dirty' work areas

The basement laboratory where excreta samples are processed should be considered in its entirety a 'dirty' area, however within this 'clean zones' should be designated for any items that will later be taken out of the laboratory:

- Sample boxes and equipment used to handle samples should only be placed on wipe-clean surfaces - plastic or metal top workbenches or trays.
- Any 'clean' items that will be taken out of the laboratory – e.g. camera and paper forms used to record results – should be kept on a clean tray or segregated clean area of the workbench.
- 'Clean' items should only be handled whilst wearing clean gloves.

5. Materials & Equipment Required

- Paper sheets for cleaning equipment and general working area
- 5ml measuring spoon/scoop (or other appropriate volume)
- Sharp knife / instrument to trim excess faeces from scoop
- 70% ethanol for disinfection of equipment, splashes and spills
- Analytical balance
- Glass dish

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6. Procedure

Method

- Clean the measuring spoon and the glass dish, and allow to dry.
- Note volume of measuring spoon used (if necessary measure volume of spoon – see Section 8 - Calibration).

- Place the measuring spoon and the glass dish on the scales, and tare the balance
- Use the spoon to scoop a sample of faeces, such that the sample completely fills the spoon. Avoid compressing the sample as much as possible.
- Wipe the bottom of the spoon, removing any excess sample.
- Trim any sample from the top of the spoon with the Stanley knife, to leave a flat surface flush with the top of the spoon.
- Place the measuring spoon on the glass dish on the scale, and record the **mass** of the sample contained in the spoon.
- Clean all equipment used and dispose of waste sample according to the faeces sample disposal SOP.

Calculation

Calculate density:

$$density = mass/volume$$

7.Calibration

- Calibration of analytical balance as indicated by manufacturer's instructions
- When a specific measuring spoon is used for the first time, the exact volume of the spoon should be determined by pipetting ethanol into it (or other suitable non-hazardous fluid with low surface tension).

Standard Operation Procedure – VIP Odour Sampling

1. Scope and Field of Application

One of the complaints / nuisances associated with VIP latrines is the odour that comes from the faecal sludge that is stored in the pit. If the compounds that cause the odour in the pits can be identified and isolated, measures to neutralize these odours can be developed.

The odours can be captured in a powder and analysed in a GC to determine the compounds that cause the smell and these can be neutralized.

2. Safety Precautions

- Wear personal protective equipment when working with VIPs
- Dispose the used gloves after completion of analysis
- Clean the hands using antiseptic soap

3. Apparatus

Figure 1 Powder carrier

Figure 2 Powder

4. Procedure

Preparation

- Open the powder carrier
- Place powder in its compartment
- Close the carrier

Sampling

- Tie string around the powder carrier
- Suspend this in the vapour space of the pit
- Leave this for a period of approximately 1 week

Post sampling

- Remove the powder from the powder carrier
- Place the powder in the vial

PREPARED BY: *Merlien Reddy*

DESIGNATION: *Senior Technician*

DATE PREPARED*: 20 June 2013*

SUPERCEDES SOP*: none*

APPROVAL OF STANDARD OPERATING PROCEDURE

PRG Head: Prof Buckley — Signature: ……

Date: ……

Author Merlien Reddy: — Signature: ……

Date: ……..

REVISED ON	REVISED BY	TITLE	REASON FOR REVISION

Introduction

Faeces can potentially host a significant number of pathogens, and as such is classified as a biologically hazardous material. Thus, faeces cannot be disposed of as normal waste. By improperly disposing faeces into normal waste bins, personnel can potentially contract disease from any pathogens present in the samples. A sluice linked to the eThekwini sewage network has been set up in the Pollution Research Group labs to allow the safe disposal of faeces and urine.

1. Safety Precautions

General

The following general safety precautions should be taken:

- Cover any small open wounds with waterproof dressings – if large open wounds then do not carry out laboratory work.
- Always use gloves, laboratory coat and closed shoes while working in the laboratory.
- Wear a face-shield when disposing of samples down the sluice (risk of splashback).
- Dispose of samples as specified by the Faeces Sample Disposal SOP.
- Clean all soiled equipment thoroughly after use.
- Any equipment that will be taken out of the laboratory into a ‘clean’ environment should be handled only with clean gloves and disinfected using 70% ethanol spray after use.
- Dispose of the used gloves in the appropriate waste bin after sample handling and disposal and cleaning of equipment is complete.

- Clean hands using antiseptic soap.
- Disinfect hands after washing with soap.

Where mixed samples are being handled (i.e. those from field location sources such as community ablution blocks), additional care must be taken as sharps may be present in the faecal matter. Samples should not be handled directly with gloved hands, but rather with a spoon or spatula.

Maintain 'clean' and 'dirty' work areas

The basement laboratory where excreta samples are processed should be considered in its entirety a 'dirty' area, however within this 'clean zones' should be designated for any items that will later be taken out of the laboratory:

- Sample boxes and equipment used to handle samples should only be placed on wipe-clean surfaces - plastic or metal top workbenches or trays.
- Any 'clean' items that will be taken out of the laboratory – e.g. camera and paper forms used to record results – should be kept on a clean tray or segregated clean area of the workbench.
- 'Clean' items should only be handled whilst wearing clean gloves.

2. Materials & Equipment Required

- Paper sheets for cleaning spilled faeces, equipment and general cleaning
- Metal/plastic spoons for scraping faeces into sluice
- 70% ethanol for disinfection of equipment, splashes and spills

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3. Procedure

All faeces must be disposed of using the sluice. The procedure for the disposal follows:

- Pour unwanted faeces samples into the sluice, and scrape and excess sample from the sample container if necessary
- Flush the sluice once all sample has been disposed of into the sluice
- Clean the sluice of any unflushed faeces
- Clean all containers and equipment used with water and dishwashing detergent, and disinfect with 70% ethanol

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DESIGNATION: *Senior Technician*

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APPROVAL OF STANDARD OPERATING PROCEDURE

PRG Head: Prof Buckley — Signature: ……

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REVISED ON	REVISED BY	TITLE	REASON FOR REVISION

1. Scope and field of application

Excreta samples are obtained from several sources including (i) segregated faeces samples from individual donors, (ii) mixed excreta samples from community ablution blocks and (iii) mixed excreta samples from household urine diversion toilets.

Incoming samples should be named according to a standard naming system and basic characteristics recorded: mass, photo record and Bristol stool classification. This SOP describes the system used for samples obtained as part of the Reinvent the Toilet project.

Human excreta can potentially host significant levels of pathogens, and as such is classified as a biologically hazardous material. Suitable precautions must be taken when handling samples.

2. Principle

Samples are named according to their source and the sequence of samples obtained from that source.

Mass measurements of the complete sample are made using an appropriate electronic balance.

The Bristol stool scale is used to class faeces samples into one of seven categories, which may be approximately related to colon transit time.

A photo record of the sample is taken to allow later correlation of measured sample properties to its initial visual appearance.

3. Safety Precautions

General

The following general safety precautions should be taken:

- Cover any small open wounds with waterproof dressings – if large open wounds then do not carry out laboratory work.
- Always use gloves, laboratory coat and closed shoes while working in the laboratory.
- Wear a face-shield when disposing of samples down the sluice (risk of splashback).
- Dispose of samples as specified by the Faeces Sample Disposal SOP.
- Clean all soiled equipment thoroughly after use.
- Any equipment that will be taken out of the laboratory into a 'clean' environment (e.g. camera) should be handled only with clean gloves and disinfected using 70% ethanol spray after use.
- Dispose of the used gloves in the appropriate waste bin after sample handling and disposal and cleaning of equipment is complete.
- Clean hands using antiseptic soap.
- Disinfect hands after washing with soap.

Where mixed samples are being handled (i.e. those from field location sources such as community ablution blocks), additional care must be taken as sharps may be present in the faecal matter. Samples should not be handled directly with gloved hands, but rather with a spoon or spatula.

Maintain 'clean' and 'dirty' work areas

The basement laboratory where excreta samples are processed should be considered in its entirety a 'dirty' area, however within this 'clean zones' should be designated for any items that will later be taken out of the laboratory:

- Sample boxes and equipment used to handle samples should only be placed on wipe-clean surfaces - plastic or metal top workbenches or trays.
- Any 'clean' items that will be taken out of the laboratory – e.g. camera and paper forms used to record results – should be kept on a clean tray or segregated clean area of the workbench.
- 'Clean' items should only be handled whilst wearing clean gloves.

5. Materials & Equipment Required

- Analytical balance for single-donor samples
- Lower sensitivity balance for large mass samples
- Permanent marker
- Camera
- Pro forma sheet for recording basic sample data
- Pen
- Paper sheets for cleaning equipment and general working area
- 70% ethanol for disinfection of equipment, splashes and spills
- Wipe-clean trays

.

6. Procedure

Sample naming system

(i) Single-donor faeces samples

Each donor is assigned a unique 3-digit donor number on registration for the sample donation programme.

Sample numbers follow this format:

Donor number –Order of sample from that individual donor

e.g. the third sample received from donor number 001 would be named 001003.

(ii) Multiple-donor excreta samples from field locations

Each field location is assigned a 2-digit location number.

Each sampling point within that location is assigned a letter.

Sample numbers follow this format:

Location number – sampling point letter - Sample number from that sampling point

e.g. fourth sample received from pedestal B in the community ablution block with location number 002 would be named 02B004

Receipt of samples

Procedure for receipt and storage of samples:

- Assign correct name to sample and write on the container with permanent marker.
- Write time sample transferred to coldroom on sample container with permanent marker.
- Store samples in coldroom (refer to Sample storage SOP).
- Record sample names and dates and times donated and transferred to storage in the sample database.

Mass measurement of complete sample

- Prior to sampling, measure and record mass of empty sample containers (including lid) using analytical balance.
- Write mass of container (units g) on the sample container using permanent marker.
- Measure mass of full sample containers using (i) analytical balance for single-donor samples and (ii) lower sensitivity balance for multiple-donor samples from field locations.
- Record mass of sample on pro forma sheet.
- Transfer paper record to sample database on lab computer.
- Save the updated database in the correct network location with an updated filename.

Photo record

- Two persons should carry out the procedure – one to handle samples and the other to handle 'clean' items (camera).
- Retrieve samples from storage and transfer to suitable work area (a wipe-clean surface).
- Sample handler opens all lids of sample containers containing samples to be photographed, place alongside sample so that sample name and dates and times are visible (if dealing with a large number of samples, work in manageable batches for the work area available – e.g. five to ten samples at a time).
- Photographer uses camera to photograph each sample, including the lid with the record of name, date and time in each shot.
- Sample handler closes all sample containers and returns to storage.
- Use 70% ethanol spray (sparingly) to disinfect camera. Place in a clean location in the upstairs area of the laboratory.
- Dispose of dirty gloves and wash hands according to standard procedure before transferring camera to office area to retrieve photos.

- Name each photo with the sample number of the relevant sample and save in the correct network location.
- Note on the sample database that a photo record exists for that sample.
- Save the updated database in the correct network location with an updated filename.

Bristol stool classification

- Retrieve samples from storage and transfer to suitable work area (a wipe-clean surface).
- Open all lids of sample containers containing samples to be classified.
- Dispose of dirty gloves and replace with clean gloves.
- Classify each sample with reference to the Bristol stool chart. Record classification alongside sample number on the pro forma sheet.
- Replace pro forma sheet in 'clean' work area before handling samples again.
- Close all sample containers and return to storage.
- Dispose of dirty gloves and wash hands according to standard procedure before transferring paper records back to the office.
- Transfer data from the paper records to the sample database on lab computer.
- Save the updated database in the correct network location with an updated filename.

Updating the sample database

- Data should be transferred from paper records to the electronic sample database as soon as possible.
- The updated database should be saved in the following location:

\\Un\.DBNPRG1_VOL1.ENG.und\PRG\PRG-RESEARCH\PROJECTS\Gates Reinvent the Toilet Challenge RN95\8 Project areas\Sample analysis and database

- The database filename should be edited to reflect the date it was saved on – e.g. a version saved on the 1 June 2012 would be saved with a filename starting with 20120601.

7. Calibration

- Calibration of analytical balance as indicated by manufacturer's instructions

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REVISED ON	REVISED BY	TITLE	REASON FOR REVISION

1. PROCEDURE FOR CHEMICAL WASTE DISPOSAL

The generation of waste when working with chemicals is normal. It is extremely important for everyone to minimize the amount of waste produced as lower waste production leads to a lower environmental impact. A few ways of achieving this is to:

• take care that you correctly make up reagents the first time.

• carry out your experiment correctly the first time so that the volume is reduced

• substitute toxic chemicals with less toxic chemicals

- **The way in which the waste is disposed off is of utmost importance. Depending on the composition of the waste, it needs to be professionally incinerated or placed in a designated landfill. Chemicals are not to be disposed of via the sewer or general solid waste system.**

The following steps must be taken when collecting and storing chemical waste prior to disposal:

- Make very sure that the bottle to be used has been thoroughly washed and dried. The bottle must be clean!
- When adding waste into a bottle that already contains waste, ensure that both wastes are compatible. The mixing of incompatible chemicals is extremely dangerous!

• Waste bottles must be properly separated out to avoid unwanted reactions in the event spills.

• The bottle must be properly labeled with t the following information regarding the waste:

a) The type of waste (composition of components must be stipulated in the case of multiple chemicals being present),

b) Name of person generating the waste

c) Date on which collection into the container started

d) Hazards of the chemical

- In an attempt to save costs, empty reagent bottles (2.5L Winchester bottles), 25L aluminium and plastic drums are used for collection and storage of waste products. While this is appreciated, following points need to be noted:
- Do not place acids or alkaline material in a metal container. Metal and glass containers should be used mainly for organic waste.
- Any container to be utilised for chemical waste storage must be thoroughly cleaned and must be free of any reagents. The solvent rinse from the container is classed as waste.
- The original label must be removed before pasting the new label on.
- The container must be kept outside in the bunded area.
- **ALL CHEMICAL OXYGEN DEMAND(COD) WASTE MUST BE POURED INTO THE MARKED 250L CONTAINER IN THE BUNDED AREA.**
- **ALL NITRATE AND PHOSPHOROUS WASTE CHEMICALS MUST BE POURED INTO LABELED 2.5L GLASS BOTTLES STORED IN THE BUNDED AREA.**
- Damaged glass is not to be thrown into the general rubbish bin. Chemical residue must first be removed and the glass disposed of into the special “broken glassware” bin. When this bin has reached it capacity it will be sealed and disposed of in the fitting manner.
- Used “sharps” (blades, scalpels, needles, etc.) must be put into a special rigid-walled container marked for these items. When these containers are three quarter full, they will be sealed and sent for incineration by the appropriate authority. Please consult the Laboratory Technician when your waste bottle is full. He will inform you of the waste collection points in the School. Under no circumstances may sharps be thrown into the general waste bins.
- Gloves contaminated with hazardous chemical substances must be placed into a separate container and marked as such. This too is not to be disposed of into the general purpose waste
- If you don’t know the correct procedure or the answer to something – then ask!
- Make sure that the laboratory is a safe place for everybody!

2. PROCEDURE FOR HAZARDOUS BIOLOGICAL WASTE DISPOSAL

- **DICARD ALL BIOLOGICAL WASTE (FEACES, WASTEWATER, VIP SAMPLES, SLUDGE) INTO THE SLUICE.**

UNIVERSITY OF KWAZULU-NATAL	***Standard Operating Procedure*** **Pollution Research Group**	**Effective Date:** **20 June 2013**	**Version: *001***
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Chemical spill in biochemistry laboratory			**Page #: *1 of* 5**

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REVISED ON	REVISED BY	TITLE	REASON FOR REVISION

1. PROCEDURE FOR SMALL CHEMICAL SPILLS

- Contain spill as best as possible using absorbent paper. If liquid has spilled from a container, return the container to the upright position to prevent further spread of the liquid.
- Notify all staff in the laboratory of the chemical spill.
- Close all drains to prevent the spill from reaching the environment.
- Switch off all electrical equipment in the vicinity of the spill.
- Cordon off the area and control access of unnecessary students.
- Assist any person that has been exposed to chemical contamination.
- First aid kit and spill kit available from Lab tech.
- Trained first aid workers are available in the department.
- The Safety Officer Dudley will report spill to EnviroServe if the spill is too big to handle ourselves.
- Clean up spill as follows:
- Put on all protective clothing, goggles and acid resistant gloves.
- Cover all wet spills with Spillow packs or paper towels
- Clean up all dry spill using the scoop.
- Try not to mix chemicals when scooping up. See list of incompatible chemicals on lab wall
- Place all dry chemicals in a sturdy plastic bag, tie with vinyl bag ties,
- and label if contents are known and place in bunded area for collection by Enviro Serve
- Pick up all broken glass using tongs and put it into the broken glass bin.
- Take note of all information on the labels from broken containers, both safety information and toxicity.

- After the Spillow packs have absorbed 10-20x their own weight, they are saturated and need to be replaced by another Spillow pack.
- Put saturated Spillow packs into plastic bags I bunded area for disposal by Enviroserve. Disposal of hazardous material are controlled by Rekha Maharaj ext 1056

2. Treatment of Contaminated Staff

In the case of serious injuries

- The treatment of serious injuries takes precedence over any other consideration.
- Call 10177 and request medical assistance.
- Advise the called assistance of the nature of the hazard, the amount of material, the chemical form of the material and any other pertinent information such as location.
- Direct someone to meet the emergency medical personnel.
- Ensure that the victim is comfortable and cannot be further contaminated by other chemicals.

In case of minor wounds not requiring hospitalization

- Get Trained First Aid worker to treat the affected person immediately.
- Wash the contaminated wound with copious amounts of warm water.
- Clean the affected area with swabs.
- Encourage minor bleeding.
- In the case of contaminated facial wounds, ensure that contamination does not spread to the mouth, ears, eyes or nasal passages.
- After decontamination, apply first aid dressing.

If the skin is intact

- It is very important that skin contamination be removed immediately.
- Early, effective removal of the contamination can help to reduce chemical exposure.
- During skin decontamination, it is important to proceed from mild treatments to harsher ones only if necessary. Abrasion or any other breaks of the skin must be avoided, as these will allow rapid penetration of the chemicals.
- Therefore, hard scrubbing is discouraged.
- Flush contaminated area with copious amounts of water.
- Exercise caution so as to not spread contamination to other areas of the body.
- Rinse thoroughly.
- Repeat wash/rinse procedure several times using a soft brush, if necessary.

Eyes, Ears, Nose, and Mouth

- Use eyewash station or shower to flush eyes, ears, and nose.
- Rinse mouth with water, but do not to swallow the water.
- Hair
- Tilt head back so water doesn't run across face.
- Be sure to close eyes and mouth during decontamination.
- Wash gently with soap and warm water for 2-3 minutes in sink and rinse well.

Treatment of Clothing Contamination

- In the event that personal clothing or lab coat becomes contaminated it is
- important that it be removed quickly to reduce the person's exposure to the chemicals.
- All contaminated clothing must be sealed in plastic bags to be removed by EnviroServe
- A full emergency shower can be used for major chemical spills.

Chemical Spill Kit

1. Drum with lid and side lever locking ring.
2. Spillow phenolic foam absorbent pack or loose PP absorbent. Can be used for all spills except nitric acid (HNO3).
3. Pair of neoprene/latex gloves.
4. Chemical resistant goggles.
5. Two thick plastic bags (100 micron thick from WasteTech).
6. Two self locking vinyl bag ties.
7. Scoop to pick up Spillow or loose PP absorbent.
8. Acid resistant lab coat/plastic apron.
9. Latex shoe covers.
10. Face shield with ratchet headgear.
11. Tongs.
12. Powdered zinc/iodine/sulphur for absorbing mercury spills.
13. Activated carbon/vermiculite for blanketing effect on both toxic and flammable spills- suppresses vapours and reduces risk of combustion and explosion.
14. Clean beach sand for acid spills.

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REVISED ON	REVISED BY	TITLE	REASON FOR REVISION

1. GASES AND LIQUIDS UNDER PRESSURE

- Permanent gases, oxygen, nitrogen, air, argon, helium, hydrogen, methane, etc. are supplied in high pressure cylinders. Numerous other gases are supplied as liquids under pressure.
- These include, ammonia, butane, carbon dioxide, chlorine, hydrogen chloride, propane, and sulphur dioxide. The pressure is dependant on the characteristics of the substance. In addition, the gases can be toxic and flammable.
- **Cylinders of flammable gases generally have valve outlets with left hand threads, while non-flammable gases have right-hand threaded outlets**.

2. STORAGE OF GAS CYLINDERS

- Due to the large amount of energy associated with compressed gases, gas cylinders can be very dangerous.
- All cylinders must be stored in a vertical position at all times, except for cylinders designed to be horizontal, e.g. ammonia or chlorine. This is to keep any liquid present out of the valve, and to protect the sides of the cylinder from shocks.
- Cylinders must also be chained at any given time. If a cylinder of compressed gas falls over and the fitting snaps off, that cylinder will propel like a rocket and can go through concrete walls. The cylinders must always be stored and used in a cool well-ventilated area away from all ignition sources. Valve caps should always be kept in place to protect the valve from damage and accidental opening.

- Cylinders containing noxious or toxic gases must be stored in a well ventilated area, and ALL cylinders must be returned as soon as they become empty. These cylinders should be kept aside from full cylinders, be clearly marked and their valves closed.

3. TRANSPORTATION OF GAS CYLINDERS

• Always read the label on a cylinder before transporting or connecting up a fresh cylinder. If the label is illegible or altogether missing, return to the supplier. It is unsafe to use a cylinder of unidentified contents.

• Cylinders must always be in an upright position and never on their side, except for cylinders designed to be horizontal, e.g. ammonia or chlorine. This is to keep any liquid present out of the valve, and to protect the sides of the cylinder from shocks.

• Ensure that cylinders are always chained to a stable object, whether in use, being stored or transported. A damaged valve on a cylinder means that the contents will exit with great force.

• Cylinders are not to be rolled or “walked”. Always use a proper trolley and ensure that the valve is protected with a valve cover during transportation. Do not transport a cylinder with the regulator still in place.

4. USING GAS CYLINDERS

Firstly, secure the cylinder to a permanent fixture such as a laboratory bench or a wall with a cylinder support bracket. Then select a regulator that is recommended by the supplier as this is compatible with the gas content of the cylinder. At no time must you attempt to use a cylinder without a regulator in place. Always ensure that the valve socket is clean, dry and free of damage before fitting the regulator. If defects are detected, return the cylinder to the supplier. Any dust or liquid may be cleared out by use of a jet of compressed air.

Do not quickly open and shut the valve of a cylinder because if you cannot close it the loud noise produced can damage your hearing. In addition you may discharge a toxic or highly flammable gas.

If a valve cannot be opened by hand, or the hand wheel supplied, the cylinder should be returned. Never hammer a cylinder valve.

To withdraw gas from a high pressure cylinder follow the procedure outlined below:

• Close off the regulator valve

• Open the cylinder valve until pressure is shown, then an extra quarter turn

• Adjust the regulator to the required pressure (or flow rate)

To shut-down the gas system:

• Turn off the gas cylinder valve

• Bleed the regulator and gas lines

• Turn off the regulator

Do not close off the regulator without shutting down the gas cylinder valve as this leaves the regulator under pressure.

If a cylinder of hazardous gas develops a leak, evacuate and seal off the area. Ensure all sources of ignition have been removed if the gas is flammable. Contact the fire department.

Beware of all the precautions when using liquefied gases or cryogenic liquids.

5. GAS REGULATORS

The primary function of a gas regulator is to reduce high pressure gas in a cylinder or process to a lower usable level as it passes from the cylinder to a piece of equipment. It is not a flow control device and is only used to control delivery pressure.

As there are various hazards associated with the use of gases, take proper precautions to assure safety in high pressure gas control. When unsure of an operation, seek the advice of an expert. Never use a regulator for a gas that it is not intended for. Only use the type of regulator appropriate for the gas in the cylinder, as interchanging these could lead to mixing reactive gases under pressure. Regulators should not be modified except by authorized personnel.

6. GASSES USED IN THE BIOCHEMISTRY LAB

Methane:Hazards

Carbon dioxide:

Nitrogen

Helium

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General Safety Rules

1. A laboratory coat and gloves must be worn at all times when work is being carried out in the biochemistry lab.
2. Do not touch any samples without wearing gloves.
3. The recommended glove is latex- powder free gloves.
4. Only closed, non porous shoes are worn in the lab. Hair should be tied back when working in the laboratory.
5. Only long pants worn in the lab.
6. Always spray 70 % ethanol on working areas, before and after use. This should be done regardless of whether someone else was using the workstation that day.
7. After finishing work in the working areas spray with 70% ethanol. Spray all items that you are removing from the counter too, in case they have come into contact with infectious material.
8. Remove coat and wash hands with antibacterial soap before leaving the lab.
9. Cell phones must not be answered in the laboratory and they should not be left on the bench top, but you may have them in your pocket.
10. Keys, jerseys and other non essential items should be left in the general office area.
11. All biological wastes should be disposed off into the sluice.
12. All glassware should be decontaminated with 2% jik solution.
13. For decontaminating pipettes 2% jik should be drawn up into each pipette after use. After standing for at least 20 minutes the pipettes can be removed from the disinfectant.
14. All items contaminated with hazardous biological agents should be autoclaved.
15. Chemicals should **not** be discarded down the sink. See SOP/disposal of chemical waste/03 for use and disposal of the hazardous chemicals which are routinely used in the biochemistry lab.

16. **Any feaces spill on the desk top must be dealt with immediately**. Isolate the area by placing paper towel over it and applying 2% jik. Leave for 20 minutes before discarding the paper towel in the bio hazardous waste bag.
17. **Do not exceed 45ml of solution in a 50ml conical or 13ml in a 15ml conical when centrifuging. This prevents leakage from tubes.**
18. Notify Laboratory Manager of any spill incidents and check with Laboratory Manager about whether you need to fill out an incident report form.
19. Address any problem immediately. Do not be afraid to make a suggestion if you think it could improve the safety or efficiency of the laboratory.

It is mandatory to wear eye safety goggles or a face shield when working with acids – there are no exceptions to this rule.

If you don't know the correct procedure or the answer to something - then ask!

Make sure that the laboratory is a safe place for everybody!

UNIVERSITY OF KWAZULU-NATAL	***Standard Operating Procedure*** **Pollution Research Group**	**Effective Date:** **20 June 2013**	**Version: *001***
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Biological spills in the biochemistry laboratory			**Page #:*1 of* 2**

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REVISED ON	REVISED BY	TITLE	REASON FOR REVISION

1. What should we do in an event of Infectious Waste Spills?

-
- Wear appropriate personal protective equipment.
- Examples: impermeable gloves (non-soak through gloves), safety glasses and a lab coat.
- Clean all spill contact areas with anti-bacterial soap or appropriate detergent.
- Disinfect thoroughly with a fresh dilute bleach solution. (1:10 with water).
- Soak up liquids with absorbent pads or paper towels.
- Place all spill clean-up in autoclave bags and autoclave before discarding.

2. What should we do if we are exposed to INFECTIOUS WASTE?

- Did infectious agents come in contact with any broken skin?
- Were the mucous membranes involved (mouth, nose, eyes)?
- Wash with anti-bacterial soap.
- If eyes were involved, flush with water for 15 minutes.
- Report to the clinic**.**

1. Scope and Application

The safe storage of chemicals is essential in order to:

- Provide effective management of chemicals,
- Lessen the risk of fire,
- Prevent accidental mixing in emergencies and
- To minimize exposure to corrosive and toxic chemicals.

The first principle of safe storage-Separate and isolate your most serious hazards. Segregate and store chemicals according to their compatibility and hazard category.

The second principle of safe storage- Store minimum quantities. The less you have – the smaller your risk.

2. Important Notes on Storage

- All chemicals should be hazard assessed, dated and labeled accordingly before storage.
- Chemicals that have more than one hazard should be should segregated by its primary hazard.
- Chemical compatibility must take preference when storing chemicals.
- Always keep copies of Material Safety data Sheets for each chemical in each store.
- All chemicals should be regularly inspected for any signs of deterioration in the quality their labels and their containers.
- Ventilation is needed for chemicals and containers that may release dangerous or damaging quantities of vapours or gasses that are flammable, corrosive, irritating or toxic.
- Do not store chemicals in direct sunlight and near heat sources as ovens, autoclaves and hot pipes.
- Ensure chemical containers are closed at all times and intact, if not replace.
- Do not store any chemicals in glass containers on the floor.
- Do not place cabinets that are used for chemical storage near an exit or an escape route.

- Do not store chemicals above eye level. If storage container were to break, its contents may spill into face and upper body.
- Do not store liquids and solids together as contamination may occur in case of spillages.
- Store chemicals in their original containers.
- Chemicals stored at the bench tops should be used frequently and kept to a minimum amount.
- Do not store chemicals in the fumehood- poor lab practice.

Table incompatibility matrix

	Simplified diagram of the incompatibilities between hazardous chemicals.				
Chemicals to be stored					
 flammables	+	-	-	O	+
 corrosives	-	O	-	O	O
 toxins	-	-	+	+	O
 noxious	O	-	+	+	+
 irritants	+	O	O	+	+

+ Compatible products

O Do not store, handle or waste together except if particular conditions are applied.
- Non compatible products.

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1. PROCEDURE FOR USE AND DISPOSAL OF HAZARDOUS CHEMICALS

- Hazardous chemicals should be disposed of appropriately.
- The following chemicals are routinely used in the Biochemistry laboratory. You should be aware of the procedures and any precautions, particularly with regard to your health. Safety measures stated should be strictly adhered to.
- All risk and safety criterion were obtained from the Sigma-Aldrich catalogue 2002-2003.

Inorganic compounds (liquids and solids)

- Many dry solid inorganic compounds are innocuous and can be placed in a garbage can in a suitably labeled container. Since it is impractical for each student to prepare a separate waste container, we provide labeled jars for unwanted compounds. Innocuous solids include: sodium bicarbonate, calcium chloride, sodium chloride, potassium chloride, sodium sulfate, and magnesium sulfate.
- Aqueous solutions of the inorganic compounds listed above are innocuous and may be sent into a sink. Aqueous solutions of sodium hydroxide, potassium hydroxide, hydrochloric acid, nitric acid, and sulfuric acid may also be sent into a sink, provided that you *test the solution with pH paper first* and establish that the solution's pH is between 5-11. If the solution's pH falls outside this range, you must partially neutralize the solution before disposal (do not simply dilute the solution with water unless a completely trivial amount of solution is involved).
- Inorganic compounds that have adsorbed organics should not be sent down the sink and should not be placed in the garbage can. In many cases, they can be left in a fume hood until the organics have evaporated (undesirable, but evaporation in the fume hood is better than evaporation in the lab or garbage) and then placed in the garbage.

Neutralization

- If liquids meet all standards for the sanitary sewer except for pH, campus employees may neutralize the solution before pouring down the drain. Use proper equipment. Goggles, gloves, apron, and hood are required. Add neutralizing agent slowly, stirring constantly. **If you are not familiar with neutralization techniques, do not attempt to neutralize solutions.** Call the Hazardous Waste Manager for assistance. **Report neutralization activities to the University Safety Manager for regulatory reporting.**

- Acidic solutions (pH <5)

  Adjust the pH to 5-9 using a dilute solution (e.g. KOH, NaOH, NaHCO3). Use a pH meter, indicator solution, or pH paper to determine the pH.

  Flush down the drain of a chemical sink with 20 volumes of cool water.

  Basic solutions (pH > 9)

  Adjust pH to 5-9 using a dilute solution (e.g. HCl, H2SO4, HNO3). Use a pH meter, indicator solution, or pH paper to determine pH.

  Flush down the drain of a chemical sink with 20 volumes of cool water.

Note: For highly concentrated acids, neutralization with a relatively dilute basic solution will take a very large volume of base and a long time. In this case, consider neutralization using a concentrated basic solution with plenty of ice for an ice bath, performed slowly, and carefully and with constant stirring. Monitor the temperature of the solution with a suitable thermometer to ensure that the solution doesn't get too hot. The same is true for neutralizing some concentrated bases.

2. Acid and Base Neutralization

2.1 Acids

If you wish to dilute an acid with water before neutralizing it with a base (e.g., sodium hydroxide, potassium hydroxide or sodium bicarbonate), always **add acid to water; *never* add water to acid**.

Perform all neutralizations within a fume hood while wearing nitrile rubber gloves, a lab coat, and eye protection.

2.1.1 Hydrochloric Acid

1. Slowly add hydrochloric acid to a container of cold water to form a 1:10 dilution of acid to water.
2. Slowly add a 1M potassium hydroxide, sodium hydroxide, or sodium carbonate solution until the pH is in the range of 6.0 to 8.0.
3. Flush down the drain with an excess of cold water.

2.1.2 Sulfuric Acid

1. Slowly add sulfuric acid to a container of ice-cold water to form a 1:10 dilution of acid to water.
2. Slowly add sodium carbonate until the pH is in the range of 6.0 to 8.0.
3. Flush down the drain with an excess of cold water.

2.1.3 Acetic Acid

1. Slowly add acetic acid to a container of cold water to form a 1:10 dilution of acid to water.
2. Slowly add a 1M solution of sodium hydroxide or sodium carbonate until the pH is in the range of 6.0 to 8.0.
3. Flush down the drain with an excess of cold water.

2.1.4 Phosphoric Acid

1. Slowly add phosphoric acid to a container of cold water to form a 1:10 dilution of acid to water.
2. While stirring, slowly add sodium carbonate until the pH is in the range of 6.0 to 8.0.
3. Flush down the drain with an excess of cold water.

2.2 Bases

2.2.1 Potassium Hydroxide

1. While stirring, slowly add potassium hydroxide into a container of ice water to form a 1:10 dilution of base to water.
2. Slowly add 1M hydrochloric acid about 1 ml at a time until the pH is between 6.0 and 8.0.
3. Flush down the drain with an excess of cold water.

2.2.2 Sodium Hydroxide

1. While stirring, slowly add sodium hydroxide into a container of ice water to form a 1:10 dilution of base to water.
2. Slowly add 1M hydrochloric acid about 1 ml at a time until the pH is between 6.0 and 8.0.
3. Flush down the drain with an excess of cold water.

2.2.3 Calcium Hydroxide

1. While stirring, slowly add calcium hydroxide into a container of ice water to form a 1:10 dilution of base to water.
2. Slowly add 1M hydrochloric acid about 1 ml at a time until the pH is between 6.0 and 8.0.
3. Flush down the drain with an excess of cold water.

2.3 Absolute Ethanol

Risk Profile R11

- Highly flammable

Safety Profile S7-16

- Keep container tightly closed
- Keep away from sources of ignition- No smoking

2.4 Isopropanol

Risk Profile R11-41-67

- Highly flammable
- Risk of serious damage to eyes
- Vapors may cause drowsiness and dizziness

Safety Profile S7-16-24-25/26

- Keep container tightly closed
- Keep away from sources of ignition- No smoking
- Avoid contact with skin
- Avoid contact with eyes
- In case of contact with eyes, rinse immediately with plenty of cold water and seek medical advice

Precautions that you must take while working with Isopropanol

- Wear goggles when aliquoting on the bench top
- Ensure you use in a well ventilated area
- Large quantities should not be disposed of down the sink. Transfer used solvent to empty Winchester bottles and mark clearly that it is isopropanol waste.

2.5 Concentrated hydrochloric acid (HCl)

Risk Profile R34-37

- Causes burns
- Irritating to the respiratory system

Safety Profile S53-26-45

- Avoid exposure – obtain special instruction before use
- In case of contact with eyes, rinse immediately with plenty of cold water and seek medical advice
- Wear suitable gloves

Precautions that you must take while working with HCl

- Wear goggles at all times when using HCl
- Do not dispose of down the sink. Transfer to the waste acids container.

2.6 Acetone

Risk Profile R11-36-66-67

- Highly flammable
- Irritating to eyes
- Repeated exposure may cause skin dryness or cracking
- Vapours may cause drowsiness and dizziness

Safety Profile S9-16-26

- Keep container in a well ventilated place
- Keep away from sources of ignition – no smoking
- In case of contact with eyes, rinse immediately with plenty of cold water and seek medical advice

Precautions that you must take while working with acetone

- Wear gloves
- Do not dispose of large volumes down the sink transfer to the non-chlorinated solvents waste disposal container.

2.7 Potassium permanganate

Risk Profile – R-8-22-50/53

- Contact with combustible material may cause fire
- Harmful if swallowed
- Very toxic to aquatic organisms
- May cause long-term adverse effects in the aquatic environment

Safety Profile – S53-60-61

- Avoid exposure – obtain special instruction before use
- This material and/or its container must be disposed of as hazardous waste
- Avoid release to the environment. Refer to special instructions/safety data sheet

Precautions that you must take while working with Potassium Permanganate

- Wear gloves
- Wear a mask when aliquoting the powder

2.8 Formaldehyde 37% solution

Risk Profile R34-40-43-23/24/25

- Causes burns
- Possible risk of irreversible effects
- May cause sensitization by skin contact
- Toxic by inhalation
- Toxic in contact with the skin
- Toxic if swallowed

Safety profile S53-26-39-45-51, 36/37

- Avoid exposure – obtain special instruction before use
- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Wear eye/face protection
- In case of accident or if you feel unwell, seek medical advice immediately (show label where possible)

- Use only in well ventilated areas
- Wear suitable protective clothing
- Wear suitable gloves

2.9 Methanol

Risk profile – R11-23/24/25-39/23/24/25

- Highly flammable
- Toxic by inhalation, in contact with the skin and if swallowed
- Toxic – danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed

Safety profile S53-7-16-43-36/37

- Avoid exposure – obtain special instruction before use
- Keep container tightly closed.
- Keep away from sources of ignition – No smoking
- Wear suitable protective clothing and gloves

Precautions that you must take while working with Methanol

- Where goggles and gloves
- Use only in a fume hood or Class II cabinet

2.10 Tris-acetate EDTA buffer

Risk Profile – R36/37/38

- Irritating to eyes, respiratory system and skin

Safety Profile – S26-36

- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Wear suitable protective clothing.

2.11 Ethidium bromide

Risk Profile – R46, 36/37/38

- May cause heritable genetic damage
- Irritating to eyes, respiratory system and skin.

Safety Profile – S45-26-22-36/37/39

- In case of accident or if you feel unwell, seek medical advice immediately (show label where possible)
- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Do not breathe dust.
- Wear suitable protective clothing, gloves and eye/face protection

Precautions you must take when working with Ethidium Bromide

- **Be extremely cautious when weighing out the powder**. It is recommended that you purchase in the liquid form. If you do weigh it out it out the balance must be transferred to a fume hood, which vents to the outdoors. Weighing should be done in the fume hood and extraction should remain on for 15 minutes afterwards. You should add liquid to powder in the fume hood.
- **Wear gloves and goggles at all times when using this substance.**

  All gels containing ethidium bromide should be discarded in a dedicated sharps container and marked clearly as ethidium bromide waste only. Waste tech will remove this.

UNIVERSITY OF KWAZULU-NATAL	*Standard Operating Procedure* **Pollution Research Group**	**Effective Date:** **20 June 2013**	**Version:** ***001***
		Reviewed by/Date:	
SOP_Safety_001 Field Collection and Storage of Samples			**Page #:** ***1 of*** **4**

PREPARED BY: *Merlien Reddy*

DESIGNATION: *Senior Technician*

DATE PREPARED*: 20 June 2013*

SUPERCEDES SOP*: none*

APPROVAL OF STANDARD OPERATING PROCEDURE

PRG Head: Prof Buckley — Signature: ……

Date: ……

Author Merlien Reddy: — Signature: ……

Date: ……..

REVISED ON	REVISED BY	TITLE	REASON FOR REVISION

1. PREPARATION FOR FIELD SAMPLING

- Prepare equipment for use, prior to the collection of samples. Comprising off adequate quantities of black refuse bags, newspaper/ paper towels, sample storage boxes, protective equipment, dust masks, safety goggles, latex powder free gloves, and lab coats for forearm and body protection and a substantial amount of alcohol for disinfection. First aid kit.
- **Fill out standard operating procedure and pin a copy on the notice board at Merlien desk.**

2. TRAVEL TO FIELD LOCATION and SAFETY

- Use roadworthy, appropriately insured vehicles. Driver to have appropriate license.
- Inform person at office of intended destination and estimated return time.(see form attached)
- Liaise with relevant officials at municipality
- Arrange introductions to caretaker and/or householder in charge of facility before starting sampling, ensure they are kept informed about activities taking place
- Use local facilitators where advised to do so by municipality

3. SAMPLES

Transport of sample from field location to lab

- Tight-fitting lids to be fitted to sample containers before being removed from the facility
- Full sample containers to be place inside bags or another tub inside vehicle.

Storage of sample in lab

- Samples to be labeled appropriately whilst in the field.
- Full sample containers to be taken from vehicle, through basement access door to lab and placed immediately into cold-room
- Fill in the cold room inventory list. Samples must be place in allocated project /student areas.
- All samples must be labeled with: Name of project, name of student, sample type, date and any other useful information.

Name/Student No/Contact details of samplers:_____

1._____

2._____

3._____

4._____

Driver:_____

Vehicle Registration Number:_____

Project:_____

Project Leader:_____

Physical Address of Sampling Area:

_____

_____

_____

_____

Depature and Estimated time of arrival:_____

Ethekwini Contact Person and number:_____

_____

Other:_____

CONTACT NUMBERS

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