


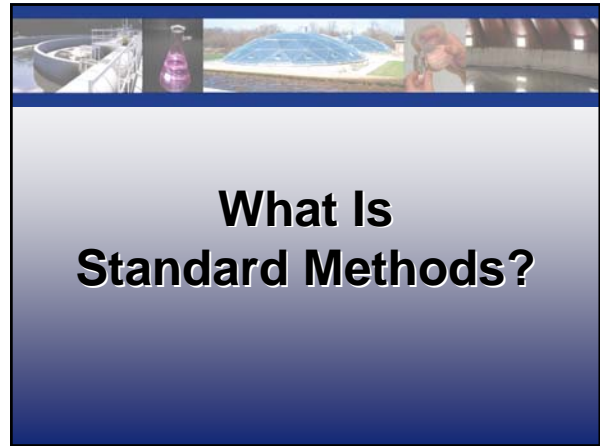
Fundamentals of Laboratory Testing

November 6, 2008

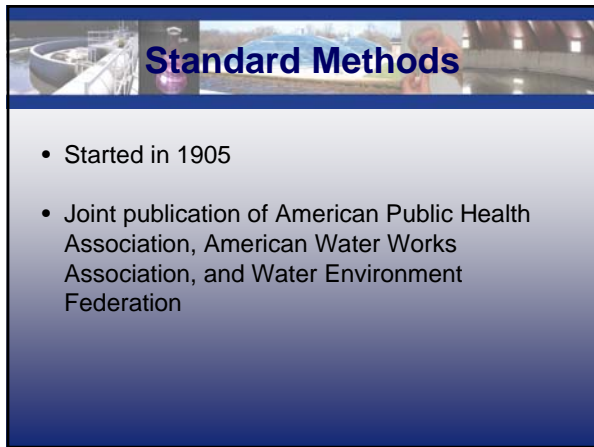
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BAXTER & WOODMAN Consulting Engineers

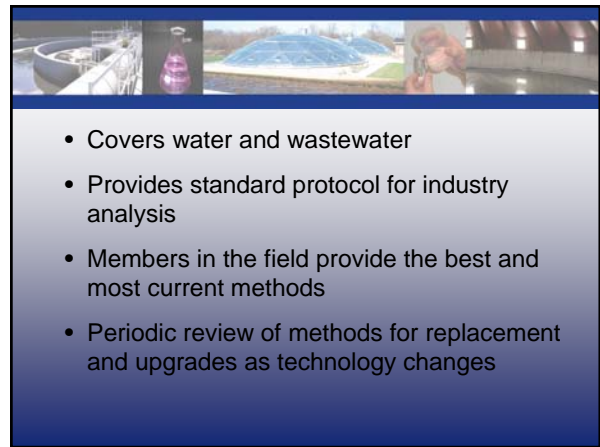


What Is Standard Methods?

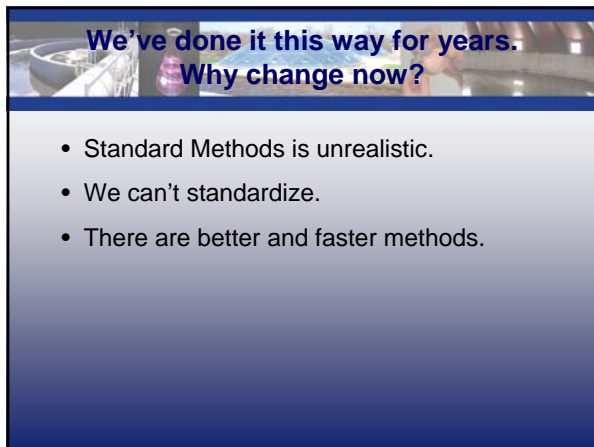


Standard Methods

- Started in 1905
- Joint publication of American Public Health Association, American Water Works Association, and Water Environment Federation

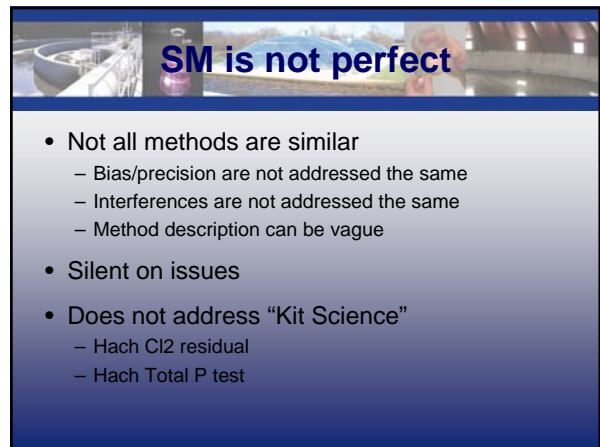


- Covers water and wastewater
- Provides standard protocol for industry analysis
- Members in the field provide the best and most current methods
- Periodic review of methods for replacement and upgrades as technology changes




We've done it this way for years. Why change now?

- Standard Methods is unrealistic.
- We can't standardize.
- There are better and faster methods.




SM is not perfect

- Not all methods are similar
 - Bias/precision are not addressed the same
 - Interferences are not addressed the same
 - Method description can be vague
- Silent on issues
- Does not address "Kit Science"
 - Hach Cl2 residual
 - Hach Total P test



Why Follow SM?

- EPA requires the use of Standard Methods
- Results are comparable when SM is used
- Creates universal process which can be reproduced
- Eliminates factors that may cause errors
- Allows user to identify issues and troubleshoot




Breakdown of Standard Methods

- Part 1000
 - Background information for analysis
 - Quality Assurance, statistics, data quality
 - Method development and evaluation
 - Expression of results
 - Sample Collection and Preservation
 - Lab Safety
 - Hazardous Waste Disposal and Minimization




Parts 2000 - 10000

2000	Physical and Aggregate Properties
3000	Metals
4000	Inorganic nonmetallic constituents
5000	Aggregate organic constituents
6000	Individual organic constituents
7000	Radioactivity
8000	Toxicity
9000	Microbiological
10000	Biological




What is in each part?

- Introduction pertaining to the type of testing
- Significance
- Types of methods
- General terms and definition
- Sampling procedures and preservation
- General precautions and interferences



Method Details

- Methods
 - Details each individual method
 - Standardization and Calibrations
 - Reagents and how to make
 - Calculations, precision and bias




GLP


Good Laboratory Practices





Safety
Equipment
Chemical Storage & Disposal



Safety



No Mouth Pipetting


PPE: Personal Protective Equipment

- Eyeglasses
- Gloves
 - Not created equal
 - Change when you spill
- Lab coats
- Safety shoes

I'm still searching for the safety sandal, but I found safety heels....



Worth 1000.com



MSDS
Material Safety Data Sheets

- Right to Know
- Need to understand your risks
- Clearly labeled book
- Look at them occasionally



Equipment



Fume Hoods

- Don't use them for storage
- Keep as free from clutter as possible
 - Creates air flow problems
- Test annually
- Keep sash in proper place



Bad



Equipment

- Instruments should be in working order
- Pay attention to details
- Frayed electrical cords
- Calibrate equipment regularly



Lack of Attention to Details



Heater/stir plate being used to stir was also accidentally turned on as hot plate!



Chemical Storage and Disposal

Chemical Inventory

- Know what you have
- Know where it is
- Dispose of old chemicals properly
- Be aware of degradation

Chemical Inventory

- Acids and Bases
 - They don't mix
 - Don't transfer to other containers
 - Keep in secondary containers
- Flammables
 - Need special cabinet
 - Separate chemicals that need to be separated



Improper Chemical Storage

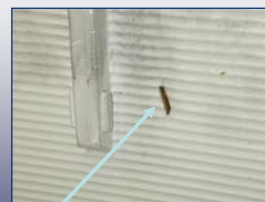
Ether was stored in this domestic-style refrigerator. Either the thermostat or the interior light was the ignition source that caused the explosion.



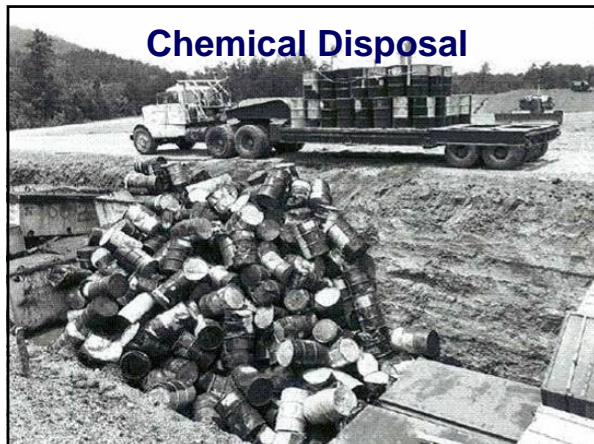
Incomplete reaction placed in refrigerator – BANG!



Reaction container was placed in the refrigerator before the reaction had been completely quenched. The bottle over-pressurized and exploded.



Note the glass embedded in the door.



Disposal

- Dispose of chemicals properly
- Read the MSDS
- Clean up spills immediately
- Clean properly

Fundamentals of Laboratory Analysis

Cleaning is important

Dishwashing

- Clean sink regularly
- Use non-phosphate detergent
- Acid wash glassware when necessary
- Rinse first with tap water, then
- Rinse 3X with distilled water

Calibrations

- Instruments should be calibrated regularly
- Balances, pipettes should be calibrated annually
- Monthly checks should be performed
 - C12
 - Balances
 - Pipettes
 - Thermometers



Temperature Control

- Temps should be recorded daily
 - Fridge/freezers
 - Water baths
 - Incubators
 - Ovens
 - Samplers
 - Muffle furnace
- Thermometers must be calibrated



Laboratory Techniques



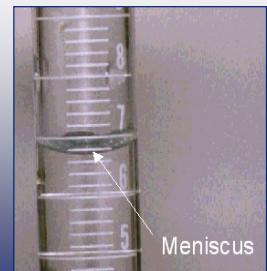
Measuring

- Use the right size
- Types of glassware
 - Class A more \$\$\$, more accurate
 - Class B less \$\$, less accurate
- To Contain or To Deliver



Meniscus

- Always read from the bottom of the meniscus
- To accurately measure, must be eye height and level



Pipetters



Pipettes

- Many styles - fixed and adjustable
- Accuracy is set by manufacturer
- Make sure tip is set right
- Draw up slowly
- Use appropriate size

Cleaning and Care

- Wipe down the pipette
- Avoid liquid in the chamber
- Disassemble the pipette and clean interior
- Reassemble and check the pipette
- Weigh DI water onto balance

Glass Pipettes

- Check for chips or cracks
- Make sure they are clean and dry
- Correct volume for the job
- Rinse after use
- Cleaning methods
- Identify type of delivery
 - To Deliver
 - To Contain
 - Wide Bore

Volumetric Pipettes

- More accurate than glass pipettes
- No chips or cracks
- Make sure they are clean and dry
- Correct volume for the job
- Rinse after use
- Cleaning methods
- Identify type of delivery
 - To Deliver
 - To Contain

Weighing

Requirements

- Solid base/stable table surface
- Level
- Out of air drafts
- Clean frequently
- Calibrate regularly
- Check monthly

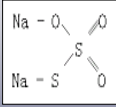
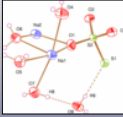


Weighing Compounds

- All Compounds are not created equal
- Check the formula
 - Water or sodium are often added to make a compound more water soluble
 - This changes the amount of the true chemical you are weighing

Sodium Thiosulfate

Added to Remove Cl_2

$\text{Na}_2\text{S}_2\text{O}_3$ 	$\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ 
<ul style="list-style-type: none"> • MW=158.11 	<ul style="list-style-type: none"> • MW=248.18



Sodium Thiosulfate

- Need to make a 1g/100mL solution
- If you use pentahydrate formula and only add 1g, result is a 0.637g/100mL solution due to the weight of the water



Sterilization

- Heat/pressure
- Irradiation
- Chemical
- Filtration (liquid)
- Most commonly use heat/pressure (autoclave)



Autoclave

- Steam under pressure
- High pressure enables steam to reach high temperatures
- Moist heat is thought to coagulate proteins, resulting in death of microorganisms



Irradiation

- Gamma rays
- X-rays
- UV



Chemical

- Ethylene Oxide (medical equipment, heat-sensitive objects)
- Ozone (water)
- Bleach
- Hydrogen Peroxide



Filtration

- For liquids
- Filters and containers are pre-sterilized
- Filter is usually 0.2 um
- Used for liquids that are sensitive to heat

Go Figure... Significant Figures

Sig Figs

- All non-zeros are significant
 - 1 has one sig fig
- Zeros appearing between numbers are significant
 - 101.23 has 5 sig figs

Sig Fig

- Leading zeros are not sig figs
 - 0.001256 has 4 sig figs
 - 1.256×10^{-3}
- Trailing zeros are
 - 7.89000 has 6 sig figs
 - 1200 has 4 sig figs
 - 0.1200 has 4 sig figs

Sig Figs

- Your accuracy is only as good as your least accurate number
 - If you are adding $18.1 + 7.9982$ you can only have 3 sig figs.
 - $18.1 + 7.9982 = 26.0982$
 - You would write this as 26.1



What Tap Water Contains

- Inorganic ions
 - Sodium, calcium, chloride, sulfate
 - Can act as catalysts
- Organics
 - Biological origin (decay, etc.)
 - Man-made (phthalate esters, chemicals)
 - Affect biological experiments

What Tap Water Contains

- Particulates and colloids
 - Soft (vegetal debris) or Hard (sand)
 - Interferes with instruments
- Bacteria and their by-products
 - Tap water is not sterile, has bacteria, viruses and by-products (pyrogens, nucleases)
 - Interferes with biological tests

What Tap Water Contains

- Gases
 - Nitrogen, oxygen, carbon dioxide
 - Affect biological reactions and instruments

Pure Water

- Ultrapure water has a resistivity of 18.31mQ
- Tap water has 15kQ
- pH of purified water is theoretically 7.0, but dissolved CO2 makes it slightly acidic

Types of Water

- Type I-analytical analysis
- Type II-what we need
 - Removes 99% of contaminants
- Type III-tap water

Water Classification

Contaminant	Parameter	Type I	Type II	Type III
Ions	Resistivity at 25°C (mega ohms-cm)	>18.0	>1.0	>0.05
	Conductivity at 25°C (microseimens/cm)	<0.056	<1.0	<20
Organics	TOC (ppb)	<10	<50	<200
Pyrogens	Eu/mL	<0.03	NA	NA
Particulates	Size	<0.2 um	NA	NA
Colloids	Silicia (ppb)	<10	<100	<1000
Bacteria	CFU/mL	<1	<100	<1000

National Committee for Clinical Laboratory Standards


Methods of Purifying Water


- Deionization
 - Uses resins that remove cations and anions
 - Does not remove uncharged molecules (virus,bacteria)
 - Use a carbon filter to remove organics
- Distillation
 - Boil water, condense steam
 - Volatiles may not be removed
 - Maintenance is high, especially in hard water
 - Copper can still leach copper
- RO, Ultrafiltration, Electrodialysis





Sample Collection and Preservation

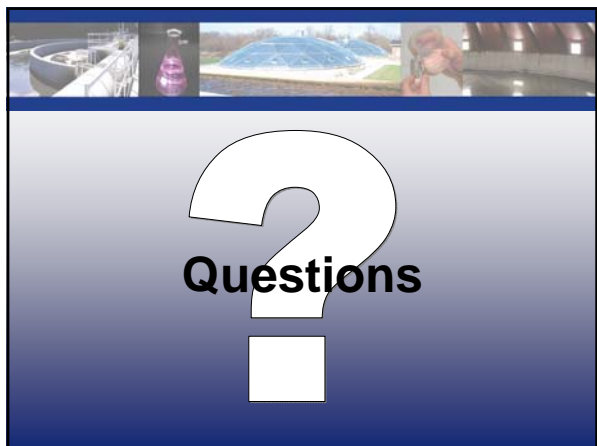
- 
- ## Sample Collection and Preservation
- Must be representative
 - Must be handled right to avoid degradation, contamination or compromising
 - Sample containers must be clean and free from interference
 - Do not pre-rinse container. This can lead to bias if compounds adhere to walls
 - If pre-filled with preservative, do not over-fill
 - Refrigeration / Freezing / Hold Time

- 
- ## Filling Container
- Biologicals need headroom
 - Volatiles should not have any headroom
 - Most other samples can have an airspace approximately 1% of total volume
 - When leaving headroom, be consistent with samples

- 
- ## Collection
- Samples need to be handled consistently
 - Same way, same time, same place
 - Label the samples
 - Add preservative immediately
 - Refrigerate/freeze immediately

- 
- ## Grab Samples
- Provide “snapshot”
 - Used when samples are not variable
 - Used for testing that requires immediate analysis
 - Cl₂, pH, temp, organics, volatiles
 - Used when samples are variable
 - Several grabs over time course to show variable
 - Do not combine samples
 - Industry

- 
- ## Composite Samples
- Represent heterogeneous matrix
 - Can be set to represent flow-based
 - Can result in loss or dilution of analyte
 - Can result in increase interferences
 - Can not be used on certain tests
 - Cl₂, pH, temp





Laboratory Compliance

By
Karen Katamay
Illinois EPA

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Types of Inspections

- There are many kinds of inspections that the Illinois EPA and USEPA do – RI, CEI, CSI, XSI, PCI, DI, LSI, PAI, etc.
- Laboratory reviews are usually only conducted during:
 - CEIs (Compliance Evaluation Inspections)
 - CSIs (Compliance Sampling Inspections)
 - PCIs (Pretreatment Compliance Inspections)
 - PAIs (Performance Audit Inspections)

2

CEIs and CSIs – lab review

- During a normal compliance inspection, things the inspector will look for are:
 - Do lab results match DMR data?
 - Do monitoring records have the required information?
 - Is laboratory equipment calibrated in accordance with manufacturer's recommendations and are records kept of this?
 - Are thermometers calibrated, or is an NIST thermometer used?
 - Is sample in accordance with permit (grab, composite, correct frequency, etc.)?

3

Lab review, cont.



- Are sampling locations representative of actual conditions?
- If automatic composite samplers are used, are temperatures checked?
- Are flow meters calibrated? If done in house, how are they calibrated?
- Does the plant collect process control data?
- Are approved methods used for sample analyses?
- Are lab records kept for a minimum of three years?

4

Your NPDES Permit

- Almost everything you need to know for compliance is in your permit:
- Sample parameters, types, frequencies, limits
 - Special sampling requirements
 - Required reports and submittal dates
 - Record keeping requirements (Std. Cond. 10)
 - Reporting requirements (Std. Cond. 12)

5



Enforcement



- Differences between civil offenses and criminal offenses
 - Effluent violations are a civil offense
 - Falsification of data is a criminal offense
 - Unless the effluent is so bad that it is an immediate threat to human health or to the environment, it usually takes more than one effluent violation to result in enforcement.
 - Effluent violations are evaluated for frequency and severity prior to any enforcement action.

6



Enforcement, cont.



- In most cases, the Agency will work with you to correct any problems your plant is having.
- NCAs (Non-Compliance Advisory letters) are sent for minor offenses.
- Violation notices are sent for more serious violations.
- Compliance commitment agreements are used for short term fixes.
- If a longer term fix is needed, compliance schedules and sometimes interim permit limits are allowed.
- Fines and penalties can sometimes be avoided if you work with us before the problems become major ones.

7

Most common issues found

- Monitoring records do not contain all the information required by Standard Condition No. 10.
- Chain of custody records for samples sent to a contract lab are not kept or are missing some of the required data.
- Samples sent to a contract lab exceed maximum holding times for parameters.
- Samples for in-house analyses (particularly pH, DO and Chlorine Residual) exceed maximum holding times.
- Samples are not properly handled or stored.
- Equipment is either not calibrated as required or there are no records of calibrations.

8

Common issues, cont.

- Samples are missing during holiday periods, or because of sick leave or vacation times.
 - Have a back up person trained to do lab operations.
 - If no back-up person, send out sample to contract lab.

9



How not to trigger an audit

- Make sure the number of samples you are taking match what is required by the permit.
- Make sure if you take more than the required amount of samples that you report all results and don't pick and choose the best data and ignore high values.
- Make sure if you have missing samples, you have records indicating why those samples are missing.
- Keep good, clean records. Erasures, numerous corrections, etc. can look like data is being changed or falsified.
- If you routinely don't properly collect or store your samples, it can invalidate your analyses.
- Make sure your personnel are properly trained in lab procedures.

10

Lab QA/QC

- Follow QA/QC recommendations per the method you are following.
- Replicates, spikes, splits, blanks, etc. need to be done at least 10% of the time or more often if required by method.
- Document inconsistencies, bad probes, etc.
- Document your SOPs.
- Document calibrations.



11

Inspection Follow-up



- Take notes on inspector's comments.
- Ask the inspector to summarize findings.
- Commit to making simple corrections ASAP.
- Give a reasonable time period for more involved corrections.
- Follow up your commitments in writing after the inspection.

12

If you have questions:

- ASK!!!! Don't wait for the next inspection to find out the hard way.
- Contact your field inspector or our office.
- Or contact our Springfield office if you have a permit or compliance question.



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Agency Contact Information

Des Plaines Office	(847) 294-4000
Springfield – Permits	(217) 782-0610
Springfield – Compliance	(217) 782-9720
Agency Laboratories	
Inorganic	(217) 278-5858
Organic	(217) 782-9780

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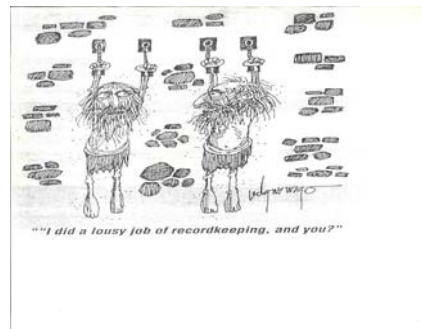
Important Websites

- Illinois EPA www.epa.state.il.us
- USEPA www.epa.gov
- Approved Methods www.nemi.gov
- IWEA (for model quality assurance plans) www.iweasite.org


The IWEA lab committee also allows you to contact them if you have lab questions at labcommittee@iweasite.org.

15


Questions????





16





Getting Great Results

- 
- Sampling
 - Standards
 - Calibration/Standardization
 - Spiked samples
 - Statistics

- 
- ## Sampling
- Required for NPDES permit
 - Influent
 - Effluent
 - Process Evaluation
 - Primaries
 - Aeration Tanks
 - Clarifiers
 - Towers
 - Sand filter
 - Digesters

- 
- ## Sampling
- Grab or Composite
 - Refrigeration
 - 4°C
 - Preserving
 - Make sure stored correctly
 - Make sure hold times are correct
 - When transferring and collecting, avoid contamination
 - Example: Fecal
 - Containers used for transport must be clean
 - No algae


- 
- ## Process Evaluation
- Various testing can tell you about problems
 - Trending can tell you about process changes
 - Sand filter not being backwashed enough
 - MLSS in aeration may signify that load has changed

- 
- ## Standards and Checks
- Standards should be run with every analysis to verify that instrument is working correctly
 - Phosphorus standards
 - Blanks/seeds/GGA
 - Fecal Blank
 - Calibrations/Standardization prepares instrument for use and to check for drift during use
 - pH
 - Ammonia
 - Balance




Standards

- Fresh standards
 - If stored, no contamination
 - Expiration date
- Avoid serial dilutions
- Mix well
- Use volumetric flasks, volumetric pipettes



Calibrations


- Instruments should be certified or checked annually
- Calibrations should be done at least monthly
 - Balance
 - Thermometers
 - Colorimeter
 - Pipettes



Spiked Samples


- Perform test on sample
- Perform test on same sample with known concentration added
- Determine recovery on spiked sample

Example:
 Phosphorus effluent sample has 0.5 mg/L P
 Spiked sample (1.0mg/L) has 1.46 mg/L P
 $(1.46-0.5)/1.0 = 0.96$ or 96% recovery




Spiked Samples

- Can tell you about possible matrix interferences
- Can tell you about sample prep or analytical procedure
- Should be chosen at random
- One bad spiked sample does not invalidate the results
- Need to review the whole to determine validity



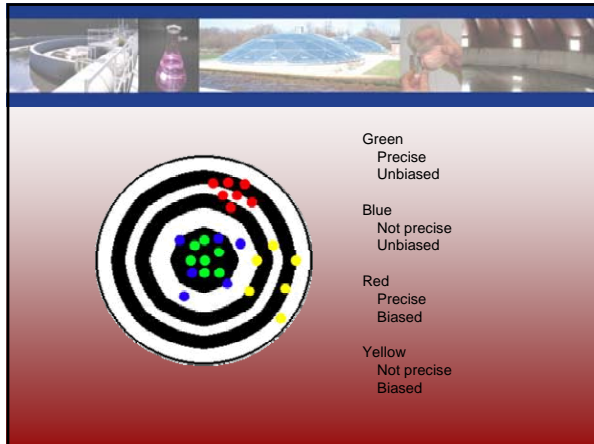
Blanks and Replicates

- Blanks
 - Zero instrument
 - Eliminates matrix issues
 - Can be a standard
- Provide quality check of reagents, solvents and system contamination
- Replicates
 - Sample
 - Blank
 - Standard
 - Spiked samples
- Checks analyst technique, system check, matrix interference, contamination, reproducibility



Precision and Bias

- Precision: how closely results are to agreeing
 - Variability
- Bias-results: closeness to true value of reference
 - Need to run several spiked samples along with replicates



Standard Deviation

- Tells you how far from the mean the discrete results are
- All are average of 7, but SD is good only for third set if the #s are very close to the average
- Bias is not addressed

Rep 1	Rep 2	Rep 3	Rep 4	Ave.	SD
0	0	14	14	7	8.08
0	6	8	14	7	5.77
6	6	8	8	7	1.54
2	9	17	0	7	7.7


What Can You Do For Your Samples?

- BE BORING!**
 - Do the same thing the same way with the same stuff
- Dedicate glassware
- Keep it clean
- Develop quality controls
- Record everything

SOPs


- Create SOPs for all procedures
 - Based on SM
- Review your procedures, techniques
- Form networks with peers to discuss troubleshooting issues, new procedures

Questions




pH

SM4500 H⁺ B




What is pH?

- pH is defined as the negative logarithm of the hydrogen ion concentration
- $\text{pH} = -\log [\text{H}^+]$




What does that mean?

- Hydrogen (H⁺) and hydroxide (OH⁻) ions are in solution
- Depending on how many of each makes a solution acidic, basic or neutral



Measuring pH

- pH 7 is neutral, equal amounts of H⁺ and OH⁻
- pH is temperature dependent, i.e.,
 - Neutral
 - 0°C pH 7.5
 - 60°C pH 6.5



Measuring pH

- Measures the hydrogen ion activity (not concentration) (ionic interactions)
- Scale is 0 (most acidic) to 14 (most basic)
- pH measures the degree of acidity or basicity of a solution
- Is a logarithmic function
- A change of one pH unit is a 10-fold change in hydrogen ion concentration




Table of Relative [OH⁻] and [H⁺] Mol/Liter Concentrations

[OH ⁻] concentration (mol/l)	pH	[H ⁺] concentration (mol/l)
1 x 10 ⁻¹⁴	0	1
1 x 10 ⁻¹³	1	0.1
1 x 10 ⁻¹²	2	0.01
1 x 10 ⁻¹¹	3	0.001
1 x 10 ⁻¹⁰	4	0.0001
1 x 10 ⁻⁹	5	0.00001
1 x 10 ⁻⁸	6	0.000001
1 x 10 ⁻⁷	7	0.0000001
1 x 10 ⁻⁶	8	0.00000001
1 x 10 ⁻⁵	9	0.000000001
1 x 10 ⁻⁴	10	0.0000000001
1 x 10 ⁻³	11	0.00000000001
1 x 10 ⁻²	12	0.000000000001
1 x 10 ⁻¹	13	0.0000000000001
1 x 100	14	0.00000000000001

Why is pH important?

- pH extremes can affect the environment of the receiving waters
- Fresh water has pH between 5 - 8.5
- Optimal pH is 6.5 - 8.2
- pH of 5 or lower is detrimental
- pH affects nutrient availability, solubility of metals and buffering capacity

How to Measure pH

- Indicators
- Colorimeters
- pH meter with glass electrode
- Titration

Indicators

- Strips of paper that change color due to pH of solution
- Wet paper with solution and compare to chart to determine pH
- Can be narrow-range or broad-range paper (0-14, 4-7, 6-8, etc.)
- Cheap, easy to use, requires low volume
- Used for approximate value, not approved for reporting purposes
- Not for continuous monitoring, easily degraded

Colorimeter

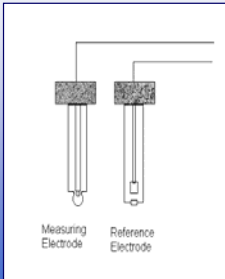
- Similar to indicators
- Reagent is added to sample
- Compare color change to chart for pH
- Can be used for grab sample but not continuous monitoring
- Interference
 - Turbidity
 - Color

pH Meter

- Precise
- Can be used for discrete sample or continuous monitoring
- Some meters can store data or download to computer
- 2 types of probes
 - Measuring probe with reference probe
 - Combination

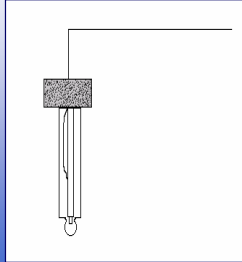
Measuring with Reference Probe

- Two probes
- Filled with buffered solution
- Measures difference in millivolts (potentiometric) across the glass membrane



Measuring with Combination Probe

- Both measuring and reference are in same probe
- Works the same way by measuring the difference across the glass membrane



Interferences

- Probe
 - Dirty probe
 - Fill solution dirty or insufficient volume
- Temperature - Affects ion dissociation and glass resistance. Causes pH shift.
 - ATC (automatic temperature compensation) probes are used to adjust for temperature differences in sample

Interferences

- Acid error - happens at pH 1 or lower. Acid displaces water. Gives higher pH reading.
- Sodium Ion – penetrates probe, replacing H ion. Gives lower pH reading.

Probe Maintenance

- Keep probe filled with KCl solution
 - Fresh KCl solution
- Do not wipe probe – Just rinse and pat dry
- Leave fill hole open (per manufacturer's instructions)
- Store probe in proper solution - dehydration will slow the response
- Chemicals will affect the life of probe
- Inspect probe periodically
 - Cracks
 - Precipitate

Standardizing Probe

- Buffers need to bracket the samples' pH
 - Common to use 4, 7, and 10
- Standards should be fresh
- Standards should be room temp
- Container needs to be large enough to allow probe to be submerged without touching walls of container

Standardization

- Beakers should be polyethylene or TFE
- Start with lowest pH standard
- Rinse probe with deionized/distilled water
- Place temp probe and electrode into solution
- Solution should be stirred gently
- Clear old standards and begin new standardization

Standards

- Slope should be between 90-102% (most common)
- Should re-standardize according to manual

Samples

- Samples must be run immediately
 - pH can change over time due to dissolved oxygen
- Record sample temps, pH, slope, time and name of analyst on bench sheet
- Samples should to be stirred gently
- Container should be polyethylene or Teflon

Results

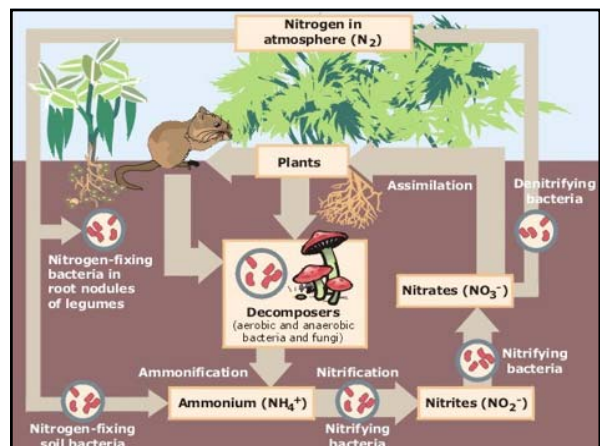
SM states, +/- 0.1 pH unit represents the limit of accuracy under normal conditions, especially for water and poorly buffered solutions. For this reason, report pH values to the nearest 0.1 pH unit


Ammonia

SM 4500 NH₃ D Ion Selective

Nitrogen Oxidation States


• Nitrate (highest oxidation)	Interconvertible
• Nitrite	Make up
• Ammonia	Nitrogen
• Organic nitrogen	cycle






Nitrate and Nitrite

- NO_3^-
- Can be high in ground water and wastewater plants
- Low in surface water
- Essential for photosynthetic autotrophs (use $\text{CO}_2/\text{bicarb}$ as C source)
- Contributes to methemoglobinemia in infants
- NO_2^-
- Formed during oxidation of ammonia to nitrate and reduction of nitrate
- Found in water systems, wastewater plants and natural waters
- Actual etiologic agent of methemoglobinemia (blue baby)



Ammonia

- Found in surface and wastewater
- Produced by deamination of organic nitrogen and hydrolysis of urea




Organic Nitrogen

- Proteins
- Peptides
- Nucleic acids
- Urea
- Synthetic organic material
- Does not include all organic nitrogen - only those in a tri-negative oxidation state




Why Test for Nitrogen

- Nitrogen is essential to plants and animals
- In waterways, too much increases algae and plankton, decreases oxygen and results in aquatic death
- Drinking water with high levels of nitrites can affect oxygen levels in humans



Ion Selective Probe

- Applicable in range of 0.03-1400 mg/L
- Dissolved ammonia is converted by raising pH above 11
- Measures via a semi-permeable hydrophobic membrane
- Ammonia diffuses across membrane



Ion Electrode Probe

- Dissolves in fill solution
- Reacts with water to form OH^- ions
- This causes pH shift, meter reads as potential difference
- For each magnitude difference (0.1 to 1; 1 to 10) mV difference should be -59 ± 4 mV



Interferences

- Volatile amines cause high results
- Metallic ions (Hg, Ag, Cu, Au, Ni, Co, Zn) complex to ammonia and cause low results
- Residual chlorine can form chloramines causing low results
- (CO₂, SO₂, H₂S) do not interfere
- Color and turbidity do not interfere



Sampling

- Composite sample - ideally flow-based
- Refrigerate at 4°C if analysis is within 24 hours
- Preserve by lowering pH to ≤ 2 with H₂SO₄ and refrigerate at 4°C (28 day hold time)



Standardization

- Standard Concentrations as follows:
 - 1000 mg/L
 - 100 mg/L
 - 10 mg/L
 - 1 mg/L
 - 0.1 mg/L



Preparation of Standard

- Use 1000 mg/L standard
- Volumetric flasks
- Rinse volumetric 3X with deionized water
- Fill volumetric with ~ 850 mL deionized water
- Using table, add standard with calibrated pipette or volumetric pipette
- QS to 1L, cap and mix well




Standard	Volume to Add
100 ppm	100 mL of 1000 ppm standard
10 ppm	10 mL of 1000 ppm standard
5 ppm	5 mL of 1000 ppm standard
1 ppm	1 mL of 1000 ppm standard
0.1 ppm	20 mL of 5 ppm standard




Avoid Serial Dilutions
as much as possible.

One little mistake
can become HUGE!




Standardization

- Inspect probe for rips in membrane
- Place 100 mL lowest standard in 150 mL beaker with stir bar
- Place on stir plate set at lowest setting
- Rinse probe and temp probe with deionized water and place into sample
- Probe should be at a 20% angle to help alleviate air bubbles




Standardization

- Begin standardization protocol for instrument
- Add 1 mL of ammonia adjuster to raise pH above 11 (amount added may depend on manufacturer's instructions)
- After stabilization, repeat with remaining standards, working toward the highest




Samples

- Dilute samples with deionized water if concentration is outside standards
- Add equal amounts of adjuster to sample as was used with standards
- Use same size beakers and stir bars
- Use lowest stir setting
- Sample and standard temperature need to be the same




Points to Remember

- Temperature must be consistent for sample and standards
- Standards must be from lowest to highest
 - Bias will result if standards are highest to lowest
- Membrane should be changed according to manufacturer's directions
 - Tracking mV readings will indicate membrane life
- Same size beakers and stir bars
- Add same amount of ISA to standards and samples



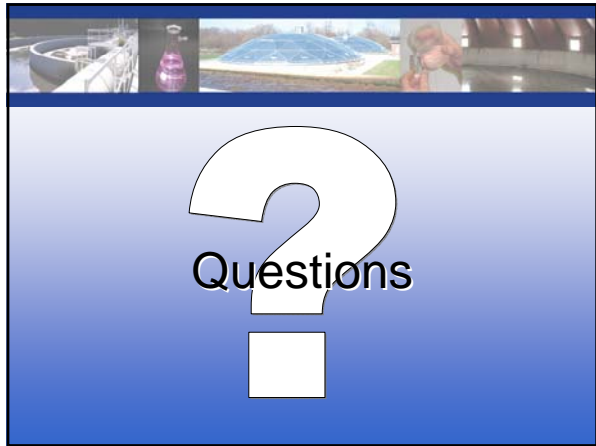
Precision and Bias

- Standard Deviation increases as concentration decreases
- Manufacturers note that bias is created if instrument is standardized with highest standards to lowest standards
- 0.03 to 1400 mg/L



Precision and Bias

- 1.00 ± 0.038 SD
- 0.77 ± 0.017 SD
- 0.19 ± 0.007 SD
- 0.13 ± 0.003 SD
- 0.10 - 96% recovery
- 0.13 - 91% recovery





Phosphorus

SM 4500 P B&E



1675

- German Hennig Brand stored 50 buckets of urine in his cellar for months hoping to turn it to gold.
- Waxy glowing goo that burst into flame
- Phosphorus



Phosphorus

- Essential nutrient for life
 - DNA: phosphate backbone
 - ATP: energy source
- Considered limiting nutrient
- In water, excess levels result in:
 - Eutrophic "Well Fed" - Hypereutrophic
 - Algae bloom
 - Oxygen depletion
 - Fish and aquatic life decrease



How it gets in the Water

- Cleaning agents
- Fertilizers from agriculture and residential
- Drinking water-added to protect pipes
- Waste from animals and people
- Food waste
- Industry



Types of Phosphates

- Organic phosphates
 - Bound to plants or animals
 - Breakdown of pesticides
 - Precipitates or in solids
- Inorganic phosphates
 - Free form, not bound to plants or animals
 - Orthophosphate is most common

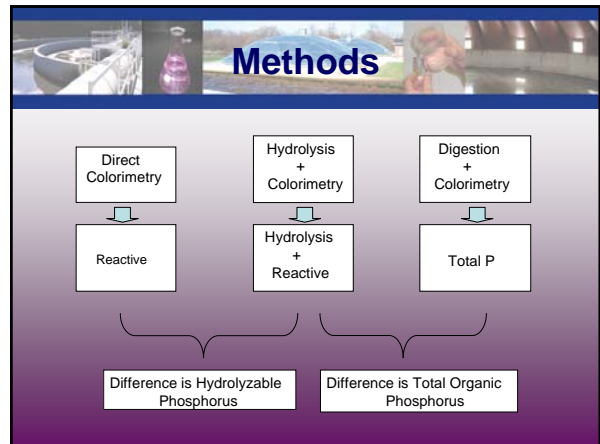


Forms of Phosphates

- Polyphosphates
- Branched polyphosphates
- Phosphoric anhydride
- Phosphite esters
- Orthophosphates
 - Reactive form

Methods of Testing

- Direct Colorimetry
 - Measures ortho or reactive phosphate
 - Will include small portion of other forms due to hydrolysis
- Hydrolysis + Colorimetry
 - Inorganic (dissolved/particles) + reactive forms
- Digestion + Colorimetry
 - Organic + inorganic + reactive



Hydrolysis

- H_2SO_4
- Converts organic condensed phosphates to ortho
 - Pyrophosphates
 - Tripolyphosphates
 - Hexametaphosphate
- Polyphosphates will need to be boiled with acid

Digestion

Converts Organic and Inorganic

- Perchloric Acid
 - Longest and drastic
 - Recommended for sediments
- Nitric Acid-Sulfuric Acid
 - Recommended for most samples
- Persulfate
 - Simplest method
 - Can be used for Total Nitrogen and Total P

Colorimetry

- Vanadomolybdophosphoric Acid
- Stannous Chloride
- Ascorbic Acid

Vanadomolybdophosphoric Acid

Orthophosphate + Ammonium Molybdate

Molybdophosphoric acid

+

Vanadium

Vanadomolybdophosphoric acid

(Yellow) read at 400, 420, 470 (conc. dependent)

Stannous Chloride

Orthophosphate + Ammonium Molybdate

↙

Molydophosphoric acid

+

Stannous Chloride

↘

Molybdenum blue
(Blue) read at 690 (aqueous) 625 (benzene-isobutanol extraction)

Ascorbic Acid

Orthophosphate + Ammonium Molybdate

↙

+ antimony potassium tartar

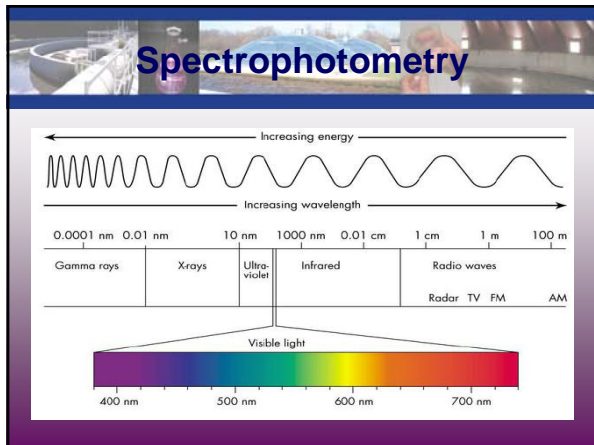
Molydophosphoric acid

+

ascorbic acid

↘

Molybdenum blue
(Blue) read at 880



- ## Spectrophotometry
- White light is made up of all wavelengths
 - Measured in nm - one billionth of a meter
 - When an object is blue, it absorbs all but the blue spectrum
 - Spectrophotometer can break down light
 - Able to deliver a narrow band (10nm) of light
 - Able to measure how much is absorbed

- ## Spectrophotometry
- We use color and intensity to measure concentration
 - Molybdenum Blue is measured at 690 or 880 because it reflects those wavelengths

- ## Sampling and Preservation
- Sampling
 - Composite sample
 - Influent, Effluent, Process points
 - If you are determining dissolved P, filter sample
 - Preservation
 - Do not add preservative if testing Reactive P
 - Add H₂SO₄ if determining Total P
 - Plastic bottles will absorb P - don't use for low levels or freeze sample
 - 28 day hold time



Sample Preparation for Total Phosphorus Digestion

SM 4500-P B



Persulfate Digestion

- Place 50 mL of sample into 150 mL erlenmeyer flask
- Add 1 drop (50 μ L) of phenolphthalein indicator
 - If it turns red add H₂SO₄ until clear, then add 1 mL more.
- Add 0.5 g solid potassium persulfate or 0.4 g ammonium persulfate
- Boil until 10 mL of solution is left (30-40 min)



Persulfate Digestion

- Cool
- Bring volume to 30 mL with distilled water
- Add 1 drop phenolphthalein indicator
- Add NaOH until pink color develops
- Bring volume to 100 mL with distilled water



Ascorbic Acid Method Colorimetry

SM 4500-P E



Reagents

- Sulfuric Acid - 5N
- Combined reagent containing
 - Antimony potassium tartrate
 - Ammonium molybdate
 - Ascorbic acid
- Phosphate standard 50 μ g/mL



Combined Reagent

- Into 100 mL flask add the following:
 - 50 mL of 5 N NaOH
 - 5 mL of 2.743 g/L antimony potassium tartrate
 - 15 mL of 40 g/L Ammonium molybdate
 - 30 mL 17.6 g/L ascorbic acid

Standards

Standard	Amount for 1L
2.0 mg/L	2 mL of 1000 mg P/L
1.0 mg/L	1 mL of 1000 mg P/L
0.5 mg/L	0.5 mL of 1000 mg P/L
0.25 mg/L	0.25 mL of 1000 P/L
0.05 mg/L	100 mL of 0.5 mg P/L
0.02 mg/L	20 mL of 1.0 mg P/L

- 1 L volumetric flasks
- Use 1000 mg P/L as concentrated standard
- Mix well
- Room temp

Calibration Curve

- Standards are run with samples
- Blank is distilled/deionized water
- Plot absorbance vs. P concentration
- Determine slope
- Must go through origin to be valid

Calibration

$Y = 0.074142X + -0.0006$

For samples, input abs as y and solve for x

$$X = \frac{Y + 0.0006}{0.074142}$$

Correlation Co. = 0.999179

Procedure

- Pipette 50.0 mL of sample or standard into 125 mL erlenmeyer flask
- Add 1 drop phenolphthalein indicator
 - If red, add 5 N H₂SO₄ drop-wise to clear
- Add 8 mL of combined reagent
- Read after 10 min (30 min max) at 880nm

Determine Concentration

- Using the equation from the calibration curve determine the concentration

$$X = \frac{Y + 0.0006}{0.074142}$$

Glassware

- Glassware should be dedicated for Phosphorus testing only
- Acid wash frequently
- Leave glassware filled with deionized/distilled water when not in use



Precautions

- P is absorbed into glass
- Careful when performing digestions Hot Acid
- Digestion should be performed in a fume hood



Interferences

- Glassware with P
- Silica and arsenate if heated
- Arsenate, Fluoride, thorium, bismuth, sulfide, thiosulfate, thiocyanate, excess molybdate
- Ferrous iron causes blue color if >100mg/L
- Hexavalent chromium and NO₂ can give low results



?
Questions



Chlorine Residual

SM 4500 Cl-G
SM 4500 Cl-C



Chlorine as a Disinfectant

- Very reactive compound
- Not instantaneous - needs time to work
- Disinfectant does not mean sterile
- Forms many different compounds



Mechanism of Disinfection

- Still researching
- Possibly:
 - Glucose Oxidation Inhibition
 - Adenine Nucleotide Depletion (ATP energy)
 - DNA Replication Inhibition



Total Chlorine

- Testing for Chlorine that has not combined with other compounds (Free Chlorine)
- Testing for chlorine that has combined to form monochloramine, dichloramine, nitrogen trichloride and other chloro derivatives



Forms of Chlorine

- Free molecular chlorine
- Hypochlorous acid HOCl
- Hypochlorite ClO⁻
- Salts - Chlorine reacts with various ions
 - Sodium, Potassium
- Ammonia – mono- and di- chloramines
- Nitrogen – nitrogen trichloride



Types of Tests

- Iodometric Method
- Amperometric Titration Method
- DPD Ferrous Titrimetric Method
- DPD Colorimetric Method
- Syringaldazine (FACTS) Method
- Iodometric Electrode Technique



DPD

SM 4500 Cl-G




Equipment

- Photometric
 - Spectrophotometer
 - 515nm, 1cm wavelength
 - Filter photometer-
 - 490-530nm, 1cm wavelength
- Glassware
- Sample vials




Reagents

- DPD pillow indicator
 - Phosphate buffer
 - DPD
 - Potassium iodide
- Chlorine standards



Sampling

- Grab sample
- Read immediately
- Do not shake
- Do not expose to light



Combined Chlorine

+

Iodide

→ Iodine

+


Free Chlorine

+

DPD


↓

Red Color




Procedure

- Place 10 mL of sample into two vials
- Blank - no DPD
- Test vial - add DPD
- Set timer
- Place blank into reader and zero
- Place sample into reader and press read




Interferences

- Blank must be same matrix to avoid erroneous readings
- Extreme acid or alkalinity
- Bromine
- Chlorine Dioxide
- Chloramines
- Iodine, Manganese, Chromium
- Ozone
- Peroxide




Limit of Detection

- Varies with instrument
- Hach 890 states 0.02 as LD




Iodometric Electrode

SM 4500 CI-I




Principle

- Chlorine reacts with iodide to form iodine
Chlorine=iodine
- Ion selective probe
 - Platinum (measures potential of iodine and iodide)
 - Iodide (measures potential of iodide)
- Measures potentiometric difference of the two to determine iodine (hence chlorine)




Equipment

- Electrodes
 - Combination electrode of platinum and iodide ion selective electrode
or
 - Two separate electrodes
- pH/millivolt meter
 - Expanded scale with 0.1 mV or direct reading selective ion meter



Reagents


- pH 4 buffer
- Chlorine demand-free water
- Potassium iodide solution
 - 42 g KI
 - 0.2 g Na_2CO_3 (sodium carbonate)
- Potassium iodate standard
 - 100 mg/L



Standards


Into each of 4 volumetric flasks, place the following and QS to 100 mL

	100 mg/L Potassium iodate Standard	Acetate buffer Solution	Potassium Iodide solution
0.2 mg/L Cl	0.2 mL	1.0 mL	1.0 mL
1.0 mg/L Cl	1.0 mL	1.0 mL	1.0 mL
5 mg/L Cl	5.0 mL	1.0 mL	1.0 mL
Blank	none	1.0 mL	1.0 mL




Standardization with Graphing

- Mix standards well
- Place into 150 mL beaker with stir bar.
- Mix gently
- Start with lowest standard
- Insert probe(s)
- Stabilize and record mV
- Repeat with remaining standards
- Using semilogarithmic paper, plot the potential (X) vs. the concentration




Standardization with Capable Meter

- Mix standards well
- Place into 150 mL beaker with stir bar.
- Mix gently
- Start with lowest standard (0.2 mg)
- Insert probe(s)
- Stabilize and record mV
- Repeat with remaining standards
- Enter standard concentration into meter




Blank

- After standardization, run the blank
- Record mV and determine concentration
- Will be used for samples with very low values (<0.2 mg/L)




Sampling

- Grab sample
- Read immediately
- Do not shake
- Do not expose to light




Procedure

- pH sample to pH 4-5 with acetic acid
- Into 100 mL volumetric flask, place:
 - 1 mL acetate buffer
 - 1 mL potassium iodide solution
- Cap. Swirl and let stand for 2 min
- Add sample to volumetric flask
- Cap. Mix by inversion and let stand 2 min




Procedure

- Pour sample into 150 mL beaker
- Immerse electrode(s)
- Stabilize reading and record mV
- For capable meter-record concentration
- For graphing-determine concentration off graph




Procedure

- If mV or concentration is above the highest standard, repeat procedure with diluted sample
- If mV or concentration is less than 0.2 mg/L, subtract reagent blank from result to obtain true value



Interferences

- Oxidizing agents
- Silver ions above 10 mg/L
- Mercuric ions above 20 mg/L
- Oxidized manganese, iodate, and copper ions




Troubleshooting Drift

- Insufficient reaction with iodide
- Oxidation of iodide
- Loss of iodine to air
- Sensing or reference element dirty




Questions



Solids


TSS
TDS
Volatiles
% Solids
Settleables

SM 2540




Types of Testing

- Settleables - SM 2540 F
- TDS - Total Dissolved Solids - SM 2540 C
- TSS - Total Suspended Solids - SM 2540 D
- Fixed and Volatile Solids - SM 2540 E
- Total, Fixed, Volatile Solids in Solids/Semisolids - SM 2540 G




What are Solids?

- What settles out
- What remains after the sample has been dried
- Total Solids refers to solids that are suspended and dissolved
- Suspended Solids are removed by settling or filtering
- Dissolved Solids do not come out of solution but are the residual left after drying the filtrate



What are Solids?

- Fixed Solids - residual that remains after ignition
- Volatile Solids - residual loss that occurs during ignition



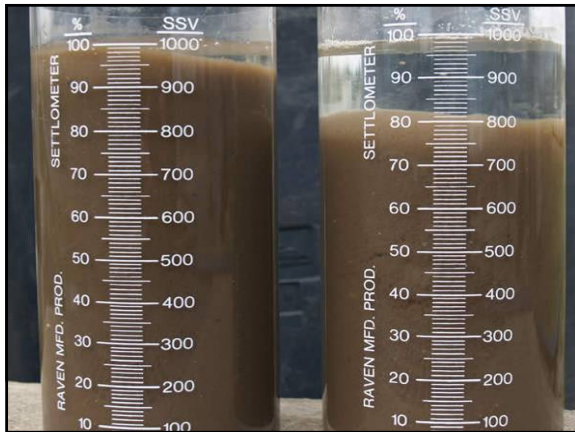
Sample and Preservation

- Glass or plastic
- Sample should not adhere to walls
- Sample should be room temp
- No preservative
- Sample should be run immediately
 - Store at 4°C for no longer than 7 days



Settleables

SM 2540 F



Settleables-Imhoff Cone

- Imhoff Cone - 1 Liter
- Mix sample by inversion
- Pour sample into Imhoff cone
- After 45 minutes, stir sides with glass rod
- After 15 minutes, read amount of solids that have settled
- Reported as ml/L

Settleables-Settleometer

- Settleometer
- Mix sample by inversion
- Pour sample into settleometer to 1000 mark
- Record the settled portion at stated intervals
- Most common time: 5 min, 15 min, 30 min, 1hr, 2hr, 4hr, 24 hr
- Reported as ml/L


Errors and Concerns

- Need care in sampling heterogeneous samples
- Mix sample prior to testing
- Note if sample adheres to wall of sample container
- If water is trapped between settled solids, subtract that volume from total

Solid Testing


Total Dissolved Solids Dried at 180°C

SM 2540 C



TDS

- Sample is filtered
- Filtrate is dried at 180°C
- Residual weight is total of dissolved solids



Equipment

- Evaporating dishes – 100 mL capacity made of porcelain, platinum, or high-silica glass
- Drying oven at 180°C or steam bath
- Desiccator
- Balance
- Stir plate and stir bar
- Wide-bore pipettes
- Graduated cylinder
- Glass-fiber filter, Whatman 934AH or equivalent
- Filtration apparatus and vacuum flask and pump




Preparation of Dishes

- Heat evaporating dishes
 - Total solids: 103°C for 1 hour
 - Volatiles: 550°C for 1 hour
- Cool and store dish in desiccator
- Tare Balance
- Weigh dish immediately before use




Preparation of Filters

- Place filter into filter apparatus wrinkled side up
 - Wash with three 20 mL volumes of water
 - Vacuum until no water remains
- Can eliminate this step if using pre-prepared filters



Testing

- Sample volume should give residual between 2.5 and 200 mg
- Sample should be stirred constantly
 - Should have vortex
- Pipette sample from middle of container, away from vortex and walls
- Add sample to filter; apply vacuum
- Wash sample 3X with 10mL DI H₂O



Testing

- Filter should be drained completely before adding each wash
- Vacuum should continue 3 min after filter is free of liquid
- Transfer filtrate to dish
- May have to add remaining filtrate in batches if volume exceeds dish volume
- Dry dish at 180°C for 1 hr



Testing

- Cool dish in desiccator
- Weigh dish
- Repeat drying, cooling and weighing until weight is constant or less than 4% of previous weight or 0.5 mg (whichever is less)



Interferences

- Hygroscopic minerals (calcium, magnesium, chloride, sulfate) may require longer drying and more care in handling
- Residual weight may include water of crystallization (in crystal structure) and occluded water (trapped)
- Occluded water loss is slow at this temp, and constant weight may take awhile



Interferences

- Samples high in bicarbonate may need longer dry time.
- Need to convert bicarbonate to carbonate for accurate weight



Equation

$$\frac{(A-B) \times 1000}{\text{Sample volume, mL}} = \text{Mg/L total dissolved solids}$$

A = weight of dish and residual, mg

B = weight of dish, mg




Precision

- Single lab analysis of 77 samples with known concentration of 293 mg/L
- SD was 21.20 mg/L




Total Suspended Solids Dried at 103°-105°C

SM 2540 D



TSS

- Sample is filtered
- Filter is dried at 103^o-105°C
- Residual weight is total of suspended solids




Equipment

- Aluminum or ceramic weighing dish or Gooch crucibles
- Drying oven at 103^o-105°C
- Desiccator
- Balance
- Stir plate and stir bar
- Wide-bore pipettes
- Graduated cylinder
- Glass-fiber filter, Whatman 934AH or equivalent
- Filtration apparatus and vacuum flask and pump
- Forceps




Preparation of Filters

- Using forceps, place filter into filter apparatus wrinkled side up
 - Wash with three 20 mL volumes of water
 - Vacuum until no water remains
- Transfer filter to aluminum dish
- Dry in oven for 1 hour




Preparation of Filters

- Cool in desiccator
- Weigh filter
- Repeat drying, cooling and weighing until filter weight difference is less than 4% or 0.5 mg
- Store in desiccator
- Can eliminate this step if using pre-prepared filters



Preparation of Gooch Crucibles

- Place filter into filter apparatus wrinkled side up
 - Wash with three 20mL volumes of water
 - Vacuum until no water remains
- Transfer filter to weighing dish
- Dry in oven for 1 hour
- Cool in desiccator



Preparation of Gooch Crucibles

- Weigh filter
- Repeat drying, cool, weighing until filter weight difference is less than 4% or 0.5 mg
- Store in desiccator
- Can eliminate this step if using pre-prepared filters



Sample Size

- Sample volume should give residual between 2.5 and 200 mg
- May need to increase sample size up to 1L
- May need to increase filter size or decrease sample size if filtration takes more than 10 minutes



Testing

- Place filter on filter holder, wet with small amount of water
- Sample should be stirred constantly
 - Should have vortex
- Pipette sample from middle of container, away from vortex and walls
- Add sample to filter; apply vacuum
- Wash sample 3X with 10mL DH_2O



Testing

- Filter should be drained completely before adding each wash
- Vacuum should continue 3 min after filter is free of liquid
- Transfer filter to aluminum dish if not using Gooch crucible
- Dry dish or Gooch crucible at 103°-105°C for 1 hour



Testing

- Cool dish in desiccator
- Weigh filter
- Repeat drying, cooling and weighing until weight is constant or less than 4% of previous weight or 0.5 mg (whichever is less)




Interferences

- Limit residual to 200 mg or less to avoid trapping water
- Residual weight may include water of crystallization (in crystal structure) and occluded water (trapped)
- Occluded water loss is slow at this temp, and constant weight may take awhile



Interferences


- Exclude large particles or materials from sample if they are not representative of sample
- Wash filter well if sample has high dissolved solids
- Do not handle dish or filters with your hands!



Equation

$$\frac{(A-B) \times 1000}{\text{Sample volume, mL}} = \text{mg/L total suspended solids}$$

A = weight of filter and residual, mg
B = weight of filter, mg



Precision


Sample Size	Standard Deviation
15 mg/L	5.2 mg/L
242 mg/L	24 mg/L
1707 mg/L	13 mg/L

Two analysts
Four sets of 10 determinations each




Fixed and Volatile Solids Ignited at 550°C

SM 2540 E




Fixed vs. Volatiles

- Ignite residual at 550°C
- Fixed is volume left after ignition
- Volatile is volume loss after ignition
- Approximate amount of organic matter present (can have vaporization of mineral salts and decomposition)
- Useful in determining process controls




Equipment

- Muffle furnace at 550°C
- Balance
- Desiccator
- Forceps
- Tongs for muffle furnace
- Samples from TDS and TSS



Procedure


- After performing TDS or TSS, place filters (in crucibles) into muffle furnace
- Include filter with no sample (blank)
- Ignite for a minimum of 20 min
- Remove samples using tongs, place on counter
- Transfer samples to desiccator until cool
- Weigh filter



Equation: Fixed Solids

$$\frac{(B-C) \times 1000}{\text{Sample volume, mL}} = \text{mg/L Fixed solids}$$


B = weight in mg of filter (crucible) and residual after ignition
 C = weight in mg of filter (crucible)



Equation: Volatile Solids


$$\frac{(A-B) \times 1000}{\text{Sample volume, mL}} = \text{mg/L Volatile solids}$$

A = weight in mg of filter (crucible) and residual *before* ignition
 B = weight in mg of filter (crucible) and residual *after* ignition



Interferences

- If volatiles are low and fixed solids are high errors will result. Use other methods to evaluate (TOC)
- Volatiles can be lost in the drying process
- Ignite blank filter in muffle furnace, if weight changes filter may not be ideal for test




Precision

- SD was 11 mg/L at 170 mg/L Volatile Solids
- Three labs, 4 samples, 10 replicates



Total, Fixed, and Volatile Solids in Solid and Semisolid Samples

SM 2540 G



What are Solid/Semisolids

- Sludge
- Sludge cakes from dewatering processes
- River and lake sediments



Equipment

- Evaporating dish
- Drying oven at 103°-105°C
- Muffle furnace at 550°C
- Desiccator
- Balance
- Stir plate and stir bar
- Wide-bore pipettes
- Forceps
- Water bath



Preparing Evaporating Dish

- For Total Solids, heat dish at 103°-105°C for 1 hour
- For Volatile Solids, heat dish at 550°C for 1 hour
- Place dish in desiccator until cool
- Weigh dish
- Store in desiccator until ready to use



TS Sample Analysis-Liquid

- If sample can flow, mix well
- Place 25-50 g in dish
- Weigh
- Place in water bath to dry
- Transfer to oven at 103°-105°C for 1 hour
- Cool in desiccator
- Weigh



TS Sample Analysis-Liquid

- Repeat heating, cooling and weighing cycle until weight change is 4% or less or weight difference is less than 50 mg (whichever is less)
- Duplicate 10% of samples
- Need to be within 5%



TS Sample Analysis-Solid

- Mix sample well with hand or take samples with #7 cork borer
- Place 25-50 g in dish
- Weigh
- Transfer to oven at 103°-105°C overnight
- Cool in desiccator
- Weigh



TS Sample Analysis-Solid

- Repeat heat (1 hr)/cool/weigh cycle until weight change is 4% or less or weight difference is less than 50 mg (whichever is less)
- Duplicate 10% of samples
- Need to be within 5%

FS vs. Sample Analysis

- Place samples in muffle furnace (cool)
- Ignite for one hour
- Cool in dessicator
- Weigh
- Repeat heat (30min)/cool/weigh cycle until weight change is 4% or less or weight difference is less than 50 mg (whichever is less)
- Duplicate 10% of samples
- Need to be within 5%

% Total Solids Calculation

$$\frac{(A-B) \times 100}{C-B} = \% \text{ Total Solids}$$

A = weight of dried residual and dish, mg

B = weight of dish, mg

C = weight of wet sample and dish, mg

% Volatile Solids Calculation

$$\frac{(A-D) \times 100}{A-B} = \% \text{ Volatile Solids}$$

A = weight of dried residual and dish, mg

B = weight of dish, mg

D = weight of residual and dish after ignition, mg

% Fixed Solids Calculation

$$\frac{(D-B) \times 100}{A-B} = \% \text{ Fixed Solids}$$

A = weight of dried residual and dish, mg

B = weight of dish, mg

D = weight of residual and dish after ignition, mg



Questions




Fecal Coliform Membrane Filter Procedure

SM 9222 D




Fecal Coliform - E. Coli

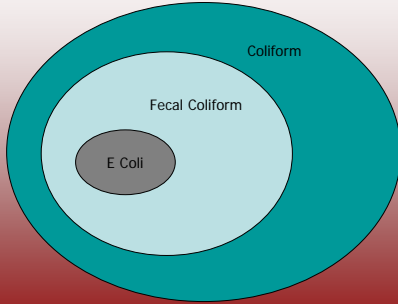


Coliform Bacteria

- Found in soil, vegetation, and intestines of warm-blooded animals
- Not all coliforms are fecal coliforms
- Not all coliforms are e.coli
- Not all fecal coliforms are e.coli
- Not all coliform, fecal coliform and e. coli are harmful





Coliform

Fecal Coliform

E Coli




Total Coliform or Fecal Coliform Testing is an Indicator Test




Fecal Coliform

- Enteric bacteria are found in intestines
- Anaerobic, gram-negative, non-spore-forming rods that ferment lactose
- 0.1% of bacteria in our gut is e.coli
- Necessary for life, provide vitamin K and B-complex




Fecal Coliform

- Able to live in environment for short time
- Good indication that water has been compromised when present
- Used to test wastewater and drinking water
- Heterotrophic (get carbon from the environment)




Types of Tests

<u>Membrane</u>	<u>Tubes</u>
<ul style="list-style-type: none"> • Petri dishes • Vacuum sample onto filter • Incubate • Count colonies • Able to give a discrete number 	<ul style="list-style-type: none"> • Liquid cultures • Inoculate broth • Change of color or turbidity or fluorescence • Tells presence or absence (MPN)




Equipment

- Autoclave
- Sterile distilled/deionized water
- Vacuum apparatus and pump
- M-FC/Rosolic acid broth
- Ampule breaker, sterile
- Sterile 50mm petri dishes with absorbent pad




Equipment

- Membranes, 47mm, 4.5 µm sterile
- Graduated cylinders
- Smooth-nosed forceps
- 70% isopropyl alcohol
- Waterbath at 44.5°C
- Whirl bags




Sampling

- Grab Sample
- Consistent collection process
 - Same time, same place, same way
- No preservative
- May have to dechlorinate with sodium thiosulfate
- Refrigerate at 4°C until analysis
- Hold time not to exceed 6 hours




Determining Dilutions

- Desired colonies per plate: 20-60
 - May need dilutions to achieve proper range
- Use range of dilutions for unknown
 - 1/10
 - 1/20
 - 1/50
 - 1/100
- Final volume should be 100mL




Procedure

- Autoclave all equipment that is not pre-sterilized
- Sterilize bench area
 - 70% alcohol
- Label petri dishes,
 - i.e., blank, 1, 2, 3 or dilution amount
- Into each dish, add 2 mL M-FC broth
 - Pour off excess broth




Procedure

- Assemble filtration device
- Place membrane on filter
- Wet to seat filter
- Blank – 100mL sterile deionized water
- Apply vacuum
- When liquid is drained, wash filter 3X with 20 mL sterile water for each rinse




Procedure

- Remove filter and place in petri dish
- Repeat procedure for remaining samples
 - May need to make dilutions
- Place petri dishes in whirl bag, invert, incubate at 44.5°C for 24 hours



Results

- After 24 hours count colonies that are blue
- Write results as per 100mL
 - Factor dilution to achieve results
- Report as Colony Forming Units/100mL (CFU)



Issues

- Chlorine residual
- Incorrect temperatures
- Sterilization
- Media problems - pH, sterilization, excess media
- Contamination during collection or preservation
- Bad technique




Questions




Biological Oxygen Demand BOD/CBOD

SM 5120B




Biological Oxygen Demand

- 5-day test
- Measures the oxygen demand
- Relates to the organics present
- Low demand = clean water
- Higher organics, higher BOD demand
- Nitrates and Phosphorus also contribute to BOD demand
 - Necessary nutrients




Organics in Water

- Organics produced by humans and industry
 - Plants, dead animals, leaves, animal waste
- Microorganisms use the oxygen in water to breakdown the organics
- Low oxygen in water results in fish and invertebrate kill
- Ammonium is also released
 - This can be converted to ammonia which is toxic




BOD vs. CBOD

BOD	CBOD
<ul style="list-style-type: none"> • Measures carbon and nitrogen oxygen demand • Measures oxygen demand to oxidize sulfides and ferrous iron 	<ul style="list-style-type: none"> • Measures only carbon oxygen demand • Add inhibitor to stop N demand



Equipment

- Dissolved oxygen meter and DO probe
- 300 mL BOD bottles
- Glass stoppers/caps
- Incubator at 20°C
- Carboy or other large (~10L) container with spigot or hose with clamp
- Graduated cylinders
- Wide-bore volumetric pipettes



Types of DO Probes

- Polarographic
- Galvanic
- Fluorescence

Polarographic DO Probe

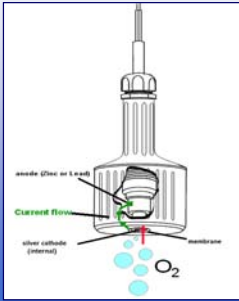
- Two electrodes
- Semi-permeable membrane
- Electrolyte (KCl)
- Anode=silver Cathode=gold
- Requires warm-up time to stabilize
- Voltage is passed through the probe
- Oxygen diffuses through membrane
- Reduced by cathode
- Produces current proportional to oxygen reduced

Galvanic DO Probe

- Two electrodes
- Semi-permeable membrane
- Electrolyte (KOH)
- Anode= Lead Cathode= Silver
- No warm-up time
- Oxygen passes through membrane
- Make their own voltage

Galvanic DO Probe

- Oxygen diffuses across membrane
- Produces electrical current
- Higher pressure allows more oxygen to diffuse
- This increases current
- Thermostat corrects for temp




Fluorescence DO Probe

- New technology to industry
- When oxygen is present, quenches emissions of fluorescent molecules in a Polymer patch
- More oxygen = more quenching
- Fiber optic cables
- Does not reduce oxygen like the other probes

Polarographic	Galvanic	Fluorescence
Temp affect		
Salinity affect	No Salinity affect	
Atmospheric pressure affect		
Reduces Oxygen	Reduces Oxygen	No oxygen reduced
Cables sensitive to movement and electrical interference Inexpensive cables Durable cables		Fiber optics cable Expensive cables Fragile cables
Membrane replaced on use and response decrease Recondition probe	Membrane replaced when response decreases	Patch lasts a long time Easy to clean and replace the patch


BOD Bottles and Glassware

- Clean glassware well. Rinse with distilled H₂O prior to use.
- Acid wash frequently.
 - Blanks with 0.2 uptake will need better cleaning or to be acid washed.
- Use correct size of graduated cylinders and pipettes.




Reagents

- Dilution Water
- Seed
- Nitrification inhibitor
- Glucose-Glutamic acid standard
- Acid or Alkali to adjust pH
- Sodium Thiosulfate - to dechlorinate



Dilution Water

- Type II water with nutrients added
 - Phosphate buffer
 - $MgSO_4$ - magnesium sulfate
 - $CaCl_2$ - calcium chloride
 - $FeCl_3$ - ferric chloride




Dilution Water

- Deionized/distilled water of Type II
- DO should be 7.5 mg/L or higher
 - Agitate to increase DO; let stand to equilibrate
- Glassware/plastic ware needs to be clean
- Free of copper, chlorine or other toxics
- Use pillow packs for ease of use
- Make sure volume is correct
- Swirl to mix
- Can store if blanks are acceptable




Seed Preparation

- Supplies microorganisms for BOD
- Commercial or make your own
- Can use settled influent or process point (clarifier, mixed liquor, undisinfectated effluent)
- Do not use any disinfected waste
- Do not filter
- Use nitrification inhibitor for CBOD results
- Can make fresh every day or create a source




Seed Preparation

- Concentration of seed controls need to meet these requirements:
- Depletion of 2.0 mg/L DO after incubation
- Residual of 1.0 mg/L DO after incubation




Nitrification Inhibitor

- Used in samples that have high oxygen demand due to nitrification microorganisms
 - Process samples (clarifiers, primaries)
 - River water
 - Raw influent
- Seed sample if nitrification inhibitor is used
- Add only after bottle is approximately 2/3 filled
- Swirl bottle to mix




Nitrification Inhibitor

- TCMP- 2-chloro-6-(trichloromethyl)pyridine
 - 3mg per 300mL BOD bottle
 - Dissolves slowly
- ATU- Allylthiourea
 - Add 0.3 mL of 2g/L solution
 - Not always effective at 2mg/L
 - Higher levels may increase CBOD
 - Higher levels may affect iodometric method




- Glucose-Glutamic acid standard
 - 150 mg/L each
- Acid and base
 - 1 N Sulfuric acid
 - 1 N Sodium Hydroxide
- Sodium thiosulfate
 - 1 g/100 mL




Samples

- Selection
- Collection
- Preparation
- Dilutions
- Seeded Samples




Sample Selection

- Influent
- Effluent
- Various process points
- Tells how the plant is performing
- Efficiency




Sample Collection

- Composite sample
- Sampler is refrigerated at 4°C
- Sample volume should be flow-based
- Sample collection should not exceed 24 hours
- Hold time begins at end of composite time
- Start analysis within 2 hours
- Can hold for 6 hours at 4°C
- Do not exceed 24 hours hold time




Sample Preparation

- Bring sample to 20±3°C
- pH of samples should be between 6.5-7.5
 - If not, adjust temp to 17-23°C or use temperature correction probe
 - pH to 7.0-7.2. Use H₂SO₄ or NaOH
 - Always seed after pH adjustment




Sample Preparation

- Dechlorinate sample if chlorine is present
 - Use $\text{Na}_2\text{S}_2\text{O}_3$
 - $\text{Na}_2\text{S}_2\text{O}_3$ will use O_2 and react with chloramines
 - Cl will dissipate in sunlight in 1-2 hours
 - Seed any sample that is dechlorinated




Sample Preparation

- Avoid shaking samples-will change the DO
 - Exceptions: supersaturated samples (cold water and high photosynthesis water) and presence of H_2O_2
- Toxic Samples
 - May require special treatment
- Hydrogen peroxide samples-agitate/shake
 - Measure H_2O_2 and DO




Sample Dilutions

- Dilutions should give DO depletion of at least 2.0 mg/L but residual of at least 1.0 mg/L
- Three dilutions per sample
 - Five if three do not give acceptable results
- Guidelines:
 - 0.1-1.0% of industrial waste
 - 1-5% for raw and settled
 - 5-25% for treated
 - 25-100% for polluted waters




Sample Dilutions

- Dilutions can be direct to bottle or prepared in other flasks
- Use class A glassware
- Use wide-bore pipettes
- Use clean glassware
- Mix sample well to avoid settling
- Use dilution water to dilute




Seeded Samples

- When to seed
 - Sample has been disinfected
 - Sample is expected to have a depletion of < 2.0mg/L
 - Standards (GGA)
- 5-day DO depletion contributed to seed should be 0.6 to 1.0 mg/L




Quality Controls

- Blanks
- Seeds
- Glucose/Glutamic acid




Blanks

- Duplicates
- Dilution water - no additions
- No nitrification inhibitor
- Measures cleanliness of bottles
- Measures quality of dilution water
- Beginning DO is ≥ 7.5 mg/L
- After 5 days, depletion should be less than ± 0.2 mg/L




Seeds

- Full strength or dilutions of seeds
- 5-day DO depletion must be > 2.0 mg/L with 1.0 mg/L remaining




QA-Glucose/Glutamic Acid

- Common standard 150 mg each of Glucose and Glutamic Acid (GGA)
- Use 6 mls of GGA (3mg/L final)
- Run with each sample set in triplicate
- Produce an average BOD of 198 ± 30.5 mg/L
- 5-day DO depletion must be > 2.0 mg/L with 1.0 mg/L remaining
- Add nitrification inhibitor if seed is nitrifying source




Filling Bottles

- Blanks
 - Dilution water / no additions
- Seed controls
 - Seed with dilution water
 - Add nitrification inhibitor if using for samples that have inhibitor
- GGA controls
 - 6 mL of 150 mg/L GGA
 - Add seed



Filling Bottles

- Samples
 - Add dilution water
 - Add nitrification inhibitor
 - If bottle is $\sim 2/3$ filled
 - Swirl to mix
 - Add sample
 - Use correct size glassware (pipette, graduated cylinder)
 - Mix sample



Initial DO Readings

- Meter should be warmed up and calibrated according to manufacturer's directions
- Insert probe, turn on stir bar
- Let reading equilibrate
- Record reading
- Record dilution volumes
- Record seed volumes

Sealing and Incubating Bottles

- Place glass stopper into bottle
- Turn stopper
- No air bubbles
- Water seal
- Secure plastic cap
- Incubate bottles in incubator at 20° C
- Allow room between bottles
- Exclude light

Final DO Reading

- Remove after 5 days ± 6 hours
- Read DO
- Record on sheet

Results

- Blanks
 - Less than 0.2 mg/L DO depletion
 - If higher depletion, dilution water or dirty glassware could be problem
- Seed controls
 - Minimum depletion of 2.0 mg/L
 - Residual of 1.0 mg/L
 - Determine DO uptake per mL (0.6 mg/L to 1.0 mg/L)
 - If higher, seed too strong
 - If lower, seed too weak, toxic condition

Results

- Glucose/Glutamic Acid
 - Results should be 198 ± 30.5 mg/L
 - If higher
 - seed too strong
 - bad dilution water
 - nitrification
 - dirty glassware
 - improper measuring techniques
 - If lower
 - weak seed
 - toxic material

Results

- Samples-No seeding
- Samples-Seeding

$$\text{BOD mg/L} = \frac{(D_i - D_f)}{V}$$

$$\text{BOD mg/L} = \frac{((D_i - D_f) - (S \cdot X))}{V}$$

Di = Initial DO
 Df = Final DO
 S = Average of seed depletion
 X = Decimal fraction of seed in bottle
 V = Decimal fraction of sample in bottle

Example-Blank

	Blank 1	Blank 2
Volume	100	100
Di	7.83	7.99
Df	7.73	7.95
difference	0.10	0.04
average	0.07	

- Blanks are good
- Depletion was less than 0.20 mg/L

Example-Seed

	Seed 1	Seed 2
Volume		
Di	7.99	7.98
Df	5.23	5.2
difference	2.76	2.78
average	2.77	

- Seeds are good
- Seed has greater than 2.0 mg/L uptake
- Residual is higher than 1.0 mg/L

Example-Final 1

	Final 1
% sample	0.1667 (50)
Di	8.05
Df	5.12
DO used	2.93
% seed	0.5 (150 ml)
DO	9.27

Seed DO	1.385
Contribution (2.77)	can not use

DO Depletion was 2.93, greater than 2.0 mg/L

Residual was greater than 1.0 mg/L

Seed contribution $2.77 * 0.5 = 1.385$

DO = 9.27
 $(2.93) - (1.385) = .1667$

Example-Final 2

	Final 2
% sample	0.3333 (100)
Di	8.09
Df	5.70
DO used	2.39
% seed	0.30 (90 mL)
DO	4.68

Seed DO	0.831
Contribution (2.77)	Valid result

DO Depletion was 2.39, greater than 2.0 mg/L

Residual was greater than 1.0 mg/L

Seed contribution $2.77 * 0.3 = 0.831$

DO = 4.68
 $(2.39) - (0.831) = .3333$

Example-Final 3

	Final 3
% sample	0.75 (225)
Di	8.04
Df	4.44
DO used	3.60
% seed	0.1 (30 mL)
DO	4.43

Seed DO	0.277
Contribution (2.77)	can not use

DO Depletion was 3.60, greater than 2.0 mg/L

Residual was greater than 1.0 mg/L

Seed contribution $2.77 * 0.1 = 0.277$

DO = 4.68
 $(3.60) - (0.277) = .75$

Example GGA

	1	2	3
GAA %	0.02	0.02	0.02
DO in	7.33	7.24	7.27
DO out	3.12	2.48	1.79
Difference	4.21	4.76	5.48
Seed %	0.1667	0.33	0.5
2.77	50 ml	100 ml	150 ml
Dil %	0.813	0.6467	0.48
DO	187	192	205

Depletion was at least 2.0 mg/L

Residual was greater than 1.0

All GGA standards are within the acceptable range of 198 ± 30.5 mg/L

Example:
 $4.21 - (2.77 * 0.1667) = 0.02$

Trouble shooting

- Rule #1 Correct your blank first
- Rule #2 To solve the problem, You will have to do extra work
- Rule #3 Change only one thing at a time
- Rule #4 Patience is a virtue

