WIL Placement Synopsis

Industry Context: My WIL placement at SAS Laboratory, a division of Urban Utilities, gave me insight into the lab's integral role within the water management ecosystem. Urban Utilities, a major water and wastewater service provider in Southeast Queensland, owns SAS Laboratory, which is designed to support water quality assurance, environmental compliance, and public health through its accredited testing services

As part of Urban Utilities, SAS Laboratory collects and analyses samples from various sectors, including **drinking water**, **wastewater**, **trade waste**, **environmental waters**, and **sewage**. The lab provides comprehensive testing services that ensure water safety and environmental protection across urban and rural areas.

Apart from microbiological testing—which involves detecting coliforms, *Escherichia coli*, and other pathogens—SAS Laboratory performs a wide range of **chemical analyses**. These include testing for **nutrient content**, such as nitrogen and phosphorus levels, as well as the presence of **heavy metals**, **pesticides**, and **hydrocarbons**. The lab also conducts tests on **airborne bacteria** and **fungi**, helping maintain air quality standards, especially in industrial and agricultural contexts.

These additional services highlight SAS Laboratory's multi-disciplinary capacity to handle various contaminants, ensuring that Urban Utilities meets environmental and public health regulations efficiently. This relationship between the lab and Urban Utilities allows for seamless integration of sampling, analysis, and reporting, making SAS Laboratory a cornerstone of water management in the region.

During my placement, I was primarily based in the microbiology department, where I worked alongside a team of microbiologists to perform various microbial tests on water samples.

These tests were critical for detecting pathogens and ensuring water safety in compliance with public health standards.

Placement Role and Responsibilities:

1. **Receiving and verifying water samples**

   Upon receiving samples, I verified the batch numbers to ensure all samples had been received and were ready for analysis.

   Why it's important:

   Verification is a critical first step in the testing process. By ensuring that each sample's batch number matches the provided documentation, we prevent mix-ups or omissions, which could lead to incorrect analysis results. This step guarantees that the samples are correctly identified and traceable, which is essential for maintaining data integrity and meeting compliance standards. Accurate sample identification is crucial, especially when dealing with thousands of samples weekly, to avoid potential errors that could compromise the results.

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2. **Preparing and labelling containers and petri dishes with batch numbers for analysis**

   After verifying the samples, I labelled two petri dishes for each sample and one 120 ml container, ensuring that the batch numbers matched the sample for easy tracking.

   Why it's important:

   Proper labelling ensures that all samples can be easily tracked throughout the analysis process. With multiple tests being conducted on each sample, it's essential that the containers and dishes are clearly labelled to prevent cross-contamination or mix-ups between samples. Labelling also helps ensure that the results correspond to the correct

sample, which is vital for accurate reporting. In a high-throughput laboratory environment, this step is key to maintaining organization, minimizing errors, and ensuring that all procedures are followed precisely according to protocol.

3. **Conducting Colilert tests by mixing media and sealing Quanti-Trays for incubation.**

   I was actively involved in microbial testing of drinking water using Colilert to detect coliform bacteria and *Escherichia coli* (E. coli). This involved measuring 100 ml of the sample into 120 ml containers and adding Colilert media. The samples were then sealed in Quanti-Trays and incubated for 18-22 hours. After incubation, results were interpreted based on color changes and fluorescence.

   Why it's important:

   Colilert tests are a rapid and reliable method for detecting coliforms and *E. coli*, which are indicators of fecal contamination in water. This step is critical for ensuring public health and safety, as the presence of these bacteria can indicate unsafe drinking water. Incubation for the appropriate time allows the media to react with the bacteria, if present, and the results provide clear visual indicators (color change or fluorescence) for quick interpretation. This method allows for timely detection, enabling swift action if contamination is found.

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4. **Performing HPC by pipetting samples onto agar plates and incubating them.**

   For HPC, I was involved in pipetting 1 ml of water samples into petri dishes, then adding Standard Plate Count (SPC) agar. These dishes were incubated for 48 hours at 37°C, after which bacterial colonies were counted to assess the microbial load in the water.

Why it's important:

The HPC method is essential for quantifying the heterotrophic bacteria present in water, which can indicate the overall microbial quality. Counting the bacterial colonies after the incubation period helps assess the microbial load and determine whether it falls within acceptable limits for safe drinking water. This process is a key aspect of water quality monitoring, ensuring that bacteria levels are not harmful to consumers and that regulatory standards are met.

5. Performing serial dilutions for highly contaminated samples

Step 1 (10^-1 dilution):

I pipetted 10 ml sample into 90 ml buffer to make (10^-1 dilution).

Step 2 (10^-3 dilution):

I pipetted 1 ml from the 10^-1 dilution into 90 ml buffer to make (10^-3 dilution).

Step 3 (10^-2dilution):

1 ml from the original into 99.9 ml buffer to make (10^-2dilution).

Why it's important:

In microbiological testing, samples often contain high concentrations of microorganisms that would result in an uncountable number of colonies on agar plates if plated directly. Serial dilutions reduce the concentration of the sample step by step, allowing you to plate a diluted sample with colony numbers that fall within the **countable range (usually 30 to 300 colonies)**. This ensures that the data collected is accurate and reliable.

6. Making buffer solutions for serial dilutions

In this procedure, I started by measuring **20 ml of monophosphate solution** and **80 ml of magnesium chloride solution**, which were mixed with **60 liters of deionized water** to form a uniform buffer solution.

Next, I measured and recorded the weights of **six empty bottles** to establish a baseline for comparison after autoclaving. The buffer solution was then poured into the bottles, ensuring an even distribution, and the bottles were placed into trays. The lids were loosely placed on each bottle to allow steam penetration during the sterilization process. **Autoclaving tape** was applied to the bottles to confirm that they had been subjected to the correct conditions.

The trays with the bottles were then placed in the autoclave, where the buffer solutions were sterilized at **121°C for 15 minutes**. After autoclaving, the bottles were re-weighed. To ensure that the correct volume of buffer solution remained in each bottle, the difference between the autoclaved bottle weight and the empty bottle weight had to correspond to a volume between **86 ml and 90 ml**. This range ensured that no significant evaporation or leakage occurred during sterilization, which could affect the buffer concentration.

After autoclaving, the buffer solution in each bottle was tested for **clarity**, ensuring that the solution remained clear and free from contamination. The **pH** of the solution was also tested to confirm that it remained within the desired range, typically around **7.2**. This step was crucial for maintaining the buffer's effectiveness. Any deviations in

clarity, weight, or pH could indicate issues with the buffer preparation or the autoclaving process.

IMPORTANCE

Weighing Bottles Before and After Autoclaving

Why it's important:

Weighing bottles before and after autoclaving helps to ensure that no significant evaporation or leakage has occurred during the sterilization process. Maintaining the correct volume of buffer solution is essential to preserving the concentration of the buffer. If too much liquid evaporates, the buffer concentration will change, potentially affecting the results of experiments that use the buffer. By ensuring that the weight difference corresponds to the expected volume of 86 ml to 90 ml, you confirm the integrity of the solution.

Autoclaving

Why it's important:

Autoclaving is critical for sterilizing the buffer solution to prevent microbial contamination, which could compromise experimental results or safety. Autoclaving at **121°C for 15 minutes** ensures that bacteria, spores, and other contaminants are killed, ensuring that the solution remains sterile and safe to use in microbiological or biochemical assays.

Testing for Clarity

Why it's important:

Testing for clarity ensures that the buffer solution is free from particulate matter, contamination, or precipitates that could interfere with its performance. A clear

solution indicates that the buffer is properly prepared and has not undergone any chemical changes or contamination during the autoclaving process. Contaminated or cloudy buffers can lead to inaccurate results in assays.

Testing pH

Why it's important:

Buffer solutions are designed to maintain a specific pH, which is crucial for many biological and chemical reactions. Testing the pH after autoclaving ensures that the buffer's pH remains within the desired range (typically around **7.2** for phosphate buffers). If the pH drifts outside of this range, the buffer may lose its effectiveness, potentially affecting the accuracy and reproducibility of experiments.

7. **Membrane filtration method**

Procedure:

Sample Collection:

In this step, I collected already verified samples that had undergone serial dilutions

Assemble the Filtration Apparatus:

I then placed sterilized funnels on the filtration apparatus in this step.

Place the Membrane Filter:

Using sterile forceps, I placed the membrane filter on the filter apparatus, ensuring that the grid side was facing up.

Filter the Water Sample:

I poured the water sample into the funnel of the filtration apparatus. Then, I applied vacuum suction to draw the sample through the membrane filter, which retained the microorganisms on its surface.

Transfer the Membrane to a Culture Plate:

After filtration, I carefully removed the membrane filter using sterile forceps. I placed the filter onto the surface of a selective or differential growth medium, ensuring there were no air bubbles between the filter and the agar. I used different types differential petri dishes to test for different organisms .

Incubate:

I incubated the plate at the appropriate temperature, usually 35–37°C for 24–48 hours, depending on the type of microorganisms I was testing for.

Examine and Count Colonies:

After incubation, I counted the number of colonies that appeared on the membrane. Each colony corresponded to a colony-forming unit (CFU), and I reported the results as CFU per 100 mL of the original water sample.

8. **SPC Media Preparation Procedure:** At the start of every week, I prepared **Standard Plate Count (SPC) media** for bacterial colony counting. The first step involved calibrating the **pH meter** to ensure accurate measurements. I calibrated the pH meter to **7.0 ± 0.2**, which is critical for maintaining the correct pH of the media. Next, I measured **434 grams of dry SPC powder**. This amount was based on the volume of media required, as it corresponds to the standard concentration for preparing the SPC media solution.

   I then mixed the dry SPC media powder with an appropriate volume of **deionized water** in a large container, ensuring thorough mixing to dissolve the powder completely. While mixing, I continuously monitored the pH, adjusting it as necessary

to maintain the pH within the **7.0 ± 0.2 range**. This ensures the media will support the proper growth of bacteria for colony counting

Once the pH was confirmed and recorded, I poured the mixture into bottles, ensuring that I mixed continuously to maintain uniformity across all the bottles. This step is crucial for preventing uneven distribution of media components, which could affect the results of bacterial growth

After filling the bottles, I placed the lids loosely to allow steam penetration during sterilization, applied **autoclaving tape** to each bottle to verify proper autoclaving conditions, and then placed them into the autoclave

The bottles were autoclaved at **121°C for 15-20 minutes** to ensure sterility, killing any microorganisms that may have been present. Autoclaving is essential to prevent contamination during the preparation of petri dishes for bacterial colony counting.

After autoclaving, the **SPC media** was analysed for **clarity, pH, and sterility** to ensure it was ready for use in microbiological testing:

Importance of checking pH, clarity and sterility

Clarity:

The media was checked to ensure it remained clear, with no cloudiness or particulates. A clear solution indicates that the media has dissolved properly and no contamination occurred during the preparation or autoclaving process.

pH:

The pH of the autoclaved media was remeasured to confirm it remained within the desired range of **7.0 ± 0.2**. Maintaining the correct pH is crucial for supporting the optimal growth of microorganisms during bacterial colony counting.

Sterility:

The media was visually inspected for any signs of contamination. Additionally, sterile techniques were followed to ensure that no microorganisms were introduced during preparation, and the autoclaving process was effective in sterilizing the media.

By analysing these factors, the SPC media was confirmed to be sterile and properly prepared for use in microbiological testing.

9. **Coliphage testing**

Bacteriophages, or phages, are viruses that specifically infect bacteria. They consist of at least a nucleic acid molecule, known as the genome, enclosed in a protein shell called a capsid. Two types of bacteriophages that target *E. coli*—somatic and F-specific coliphages—have long been used in academic research as indicators of fecal and viral contamination. Their widespread use is due to the high concentration of coliphages found in raw wastewater and other materials contaminated with fecal matter. These phages are favored for their easy, fast, and cost-effective detection and quantification methods, as well as their persistence in water environments and resistance to treatments designed to remove viruses, making them reliable surrogate indicators for various applications.(Jofre et al., 2016)

SAS sometimes receive samples that require coliphage testing , I on some days of this tests I got involved and got to learn .

Although I did was not directly involved in this test because of its complexity , I was partially involved in the test and provided some assistance were necessary.

Day before processing

On the day before processing ,the scientist I was working with had prepared overnight cultures and placed in water bath-7 overnight

Morning of processing (9 am)

Scientist had prepared log-phase host cultures and placed in water bath -7, turned on water bath -8 ,and checked for antibiotics levels.

Morning of processing (11 am)

I assisted in melting agar and placing in water bath-8 ,then I set up and labelled plates I observed scientist measure 100 ml sample and placing in 44.4$^{o}{}_{C}$ water bath for 3 minutes then add 5ml $CaCL_2$ and 5 ml host culture I assisted adding 1 ml antibiotic then combined agar and sample into a large petri dish,this was then incubated inverted into large petri dishes at 36.5 oC for 16-24 hours.

Preparation of Overnight Cultures (Day Before Processing)

Reason: Overnight cultures are prepared to grow the host bacteria (usually *E. coli*) that will later be used to support coliphage replication. This ensures that the bacteria are in an optimal phase of growth (log phase) during the test, which is critical for detecting coliphages that can infect and lyse the bacteria.

Preparation of Log-Phase Host Cultures (9 AM)

Reason: The host bacteria must be in the log phase (active growth phase) to ensure that the phages have a suitable environment to infect the bacterial cells. During this phase, the bacterial cells are most susceptible to infection by coliphages, as they are rapidly dividing.

Turning on Water Bath-8 and Checking Antibiotic Levels

Reason: Water baths are used to precisely control the temperature at different stages of the test. The correct temperature is crucial for the optimal growth of bacteria and proper coliphage activity. Antibiotics are often added to suppress the growth of other bacteria that might contaminate the test, ensuring that only the host bacteria and phages are involved.(Jofre et al., 2016)

Melting Agar and Placing in Water Bath-8 (11 AM)

Reason: Agar is melted to form a medium that can be poured into petri dishes to solidify. This creates a stable surface for bacteria and phages to grow. Keeping the agar at the right temperature in the water bath ensures it remains liquid but cool enough to not kill the host bacteria or phages when it's added to the sample.

Setting Up and Labelling Plates

- **Reason**: Proper labelling of plates is important for identifying samples and preventing mix-ups during analysis. Plates must be labelled before adding any samples to ensure clarity and accuracy throughout the experiment.

6. Placing 100 ml Sample in a 44.4°C Water Bath for 3 Minutes

Reason: This step is often used to equalize the temperature of the sample to the optimal range for coliphage activity. The specific temperature (44.4°C) and duration (3 minutes) may be chosen to ensure that any unwanted bacteria are minimized while allowing the phages to remain active for infection.(Jofre et al., 2016)

Adding 5 ml of $CaCl_2$

Reason: Calcium chloride ($CaCl_2$) is often added to enhance the adsorption of phages to bacterial cells. Calcium ions help stabilize the interaction between the coliphages and the host bacteria's cell walls, increasing the likelihood of successful infection.

Adding 5 ml Host Culture

Reason: The host culture (typically *E. coli*) is added to provide a susceptible bacterial population for the coliphages to infect. Without this step, the phages would have no host to replicate within, rendering the test ineffective.(Jofre et al., 2016)

Adding 1 ml Antibiotics

Reason: Antibiotics are included to prevent the growth of non-target bacteria. This ensures that only the target host bacteria and phages are present, preventing contamination and ensuring the accuracy of the results.

Incubating at 36.5°C for 16-24 Hours

Reason: Incubating the plates at 36.5°C provides the optimal temperature for the growth of the host bacteria and the replication of coliphages. The incubation period of 16-24 hours is chosen to allow enough time for plaques (clear zones) to form if coliphages are present. The plates are inverted to prevent condensation from dripping onto the agar, which could disturb the bacterial growth.

Deliverables: Key deliverables during my placement included:

1. **Successful daily testing and analysis of water samples using Colilert and HPC methods.**

   Other than doing usual tests I did a report on the following topic: ANALYSIS OF ENZYME SUBSTRATE COLIFORM TEST RESULTS AT SAS LABORATORY: JULY TO SEPTEMBER 2024

   This report provided an analysis of microbial water testing results obtained at SAS Laboratory between July and September 2024, using the Enzyme Substrate Coliform Test. The focus is on detecting total coliforms, indicated by a yellow appearance, and Escherichia coli (E. coli), identified by fluorescence, in water samples.

   The report highlighted key findings from positive wells and identifies operational challenges such as sample contamination and procedural errors. Additionally, it offers recommendations for improving the accuracy and efficiency of water testing at SAS Laboratory.

   From the results obtained, there were three instances of incorrect results. Two of these involved forgetting to add Colilert media to control samples, and the third resulted from the incorrect preparation of a control sample, leading to inaccurate results. While most samples were satisfactory, a few exhibited high levels of E. coli and coliforms.

2. **Development of an inventory management system**

   During my placement at SAS Laboratory, I noticed that the absence of a proper inventory management system led to stock shortages of critical supplies. Some of the items that frequently ran out included Colilert media, in-house items such as buffer media, pipette tips, and SPC agar, among others. These shortages disrupted testing procedures, causing delays and inefficiencies.

   While I was unable to develop a computer-based inventory management system during my placement, I did take the initiative to implement a **manual inventory**

system. This system involved keeping detailed logs of supplies, tracking stock levels regularly, and setting manual reorder points to prevent future shortages. Although not automated, this manual approach helped maintain better control over stock levels and reduced the frequency of running out of essential items. Introducing an automated, computer-based system in the future would greatly enhance efficiency and further prevent disruptions.

Sample of inventory system developed

Item ID	Item Name	Category	Stock Level	Reorder Level	Supplier	Expiration Date	Reorder Alert
1	Colilert Media	Media					
2	SPC Agar powder	Reagent					
3	Petri Dishes	Consumables					
4	Pipette Tips	Equipment					
In house reagents							
item id							
1	blue cap buffer						
2	white cap buffer						
3	spc agar						

3. **Development of a routine for efficiently processing samples while minimizing errors such as missed media preparation in control samples**.

During tests it was noted that some errors were being made as a result of missing some procedural steps, one example included not adding Colilert media in the control samples on two different occasions .Another example included a false result in a positive control E.coli test due to wrong preparation of the control sample.In

discussions with the lead microbiologist, we came up with routine procedures to be used in the lab to prevent such errors.

Recommendations

A. It was established that a roster be created for preparation of control samples, this was to create accountability and allow for double checking of procedures involved in making control samples .Having specific people rostered to make control samples would be crucial in minimising errors.
B. Establishing a unique procedure to be followed by all scientists .I noted that some errors like failing to add reagent media in samples may have resulted from different scientists following different procedures. In discussions with team , we came up with set procedures to be followed by everyone when performing Colilert test.
    i. In the morning before receiving samples, the team member on roster will make the control samples and another team member will verify this.
    ii. The control samples will be placed on the bench alongside with reagent media
    iii. Team member performing Colilert test will first mix powder reagent with controls before performing tests on the rest of the samples

4. I developed a Quality assurance log to be used in preparation and testing of the positive controls as follows

Date: 22nd October 2024
Activity Description: Routine *Escherichia coli* and coliform detection using Colilert media.
Personnel Involved:
Test Type: Positive and negative control for Colilert media
Controls:**Ec13 10^-8+5ML Kpa10^-8, 10ml pa 36 10^-6 , blank**
Positive control: *:Ec13 10^-8+5ML Kpa10^-8*
Negative control: Sterile water

Results:
Positive control: Yellow wells and blue fluorescence observed as expected for *E. coli* presence.
Negative control: No color change or fluorescence observed, confirming absence of contamination.
Observations: .
Corrective Actions:
Reviewed By: QA Officer – Jane Doe
Next Review Date: 25th October 2024

Through my deliverables, I contributed to the lab's mission of safeguarding public health by ensuring water quality standards were met. This placemIent not only enhanced my technical skills but also improved my ability to manage tasks under strict timelines and reinforced the importance of precision in laboratory work.