

**School of Environment and Science
Griffith University**

7105ESC_P1&2 – Work Integrated Learning Placement

ANALYSIS OF ENZYME SUBSTRATE COLIFORM TEST RESULTS AT SAS LABORATORY: JULY TO SEPTEMBER 2024

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22/10/24

SAS LABORATORY

A report submitted in partial fulfilment of the degree of Masters of Biotechnology

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1 BACKGROUND AND CONTEXT

This report provides 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 highlights 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 INTRODUCTION

Purpose of the Report

This report analyzes the results from water samples tested at SAS Laboratory using the Colilert system between July and September 2024. It focuses on the number of positive wells for coliforms and *E. coli* and discusses challenges faced during the testing period. Based on the findings, recommendations are provided to improve water testing processes at SAS Laboratory.

Background

Water is an essential resource for human survival, yet it also serves as a significant vector for the spread of diseases. Ensuring the safety of drinking water is a major public health issue. Studies indicate that 4.6% of the global disability-adjusted life years (DALYs) and 3.3% of worldwide deaths are linked to poor water quality (Wen et al., 2020a).

In addition, approximately half of the world's diseases are the result of contaminated water, and disputes over water rights have been – and remain – a global issue, as water shortages frequently lead to food scarcity and challenges in industrial production (Scott, 2013).

Microbial contamination accounts for the majority of the health burden associated with water-related issues. The World Health Organization (WHO) advises that the microbial quality of

drinking water should be assessed by measuring faecal indicator bacteria, with *Escherichia coli* being the preferred choice. These bacteria serve as indicators of faecal contamination rather than directly detecting specific pathogens.(Bain et al., 2012)

Ensuring the microbial safety of drinking water is a critical public health concern. SAS Laboratory employs the Enzyme Substrate Coliform Test to detect coliforms and *E. coli*, two important indicators of water quality. The Colilert test is a common method used for detecting total coliforms and *Escherichia coli* (*E. coli*) in water samples. It is based on the Defined Substrate Technology (DST), where the presence of these bacteria is indicated by a colour (Wen et al., 2020b)and fluorescence change in the testing media.

Enzyme Substrate Tests: Colilert

One popular approach for identifying coliforms and *E. coli* in water is the Colilert test, which uses an enzyme substrate. The presence of enzymes, β -galactosidase and β -glucuronidase, which are generated by coliforms and *E. coli*, respectively, is necessary for this test. The presence of the target organisms is shown by a colour shift or fluorescence that these enzymes create when they react with the substrates in the Colilert reagent (Fricker&Pedley,1996).

Colilert's simplicity, which requires little equipment and technical know-how, makes it especially useful for field testing. The test yields faster findings than conventional culture-based techniques and can identify *E. coli* and coliforms in as little as 24 hours. Because of its efficiency in field settings, Colilert is a well-liked option for water testing in isolated or resource-constrained locations without easy access to laboratory facilities. Colilert provides higher sensitivity and specificity for the detection of *E. coli* and coliform when compared to techniques such as membrane filtration and HPC. For thorough evaluations of water quality, other testing techniques may be required as it might not be as successful in identifying other waterborne diseases (Romper et al.,1996).

How the Colilert Test Works:

1. Sample Preparation:

- 100 ml water sample is mixed with the Colilert reagent, which contains two nutrient-indicating substrates:

- **ONPG (ortho-Nitrophenyl- β -D-galactopyranoside)** for total coliform detection.
- **MUG (4-methylumbelliferyl- β -D-glucuronide)** for *E. coli* detection.
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2. Incubation:

- The sample is incubated at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 18 to 22 hours.

3. Results Interpretation:

- **Coliform Detection:** If total coliforms are present, the ONPG is broken down by the enzyme β -galactosidase (produced by coliforms), causing the media to turn **yellow**.
- ***E. coli* Detection:** If *E. coli* is present, the MUG is broken down by the enzyme β -glucuronidase, producing a **blue fluorescence** under UV light (365 nm).
- Negative results for coliforms will remain clear, and no fluorescence will indicate the absence of *E. coli*.

3 METHODOLOGY

Each day, drinking water samples from various locations within Brisbane municipality were received and verified. The samples were then prepared for testing using 120 ml containers for Colilert tests and two petri dishes for hydrophilic plate counts (HPC). Both the containers and petri dishes were labeled with matching batch numbers corresponding to the original sample containers.

For the Colilert test, 100 ml of the water sample was poured into the 120 ml containers. Colilert powder media was added to the sample and mixed thoroughly until dissolved. The sample was then transferred into Quanti-Trays, sealed, and placed in an incubator for 18 to 22 hours.

In addition to the test samples, control samples were prepared using *E. coli*, *Pseudomonas aeruginosa*, and sterile water (blank control). These control samples were incubated alongside the test samples to ensure the accuracy and reliability of the results.

After the three months , July ,August, September, results obtained from positive tests were obtained and analyzed.

4 RESULTS AND ANALYSIS

Positive results for coliforms were indicated by yellow wells, while *E. coli* was detected through fluorescence under UV light.

Total Coliforms (Yellow Appearance) and Escherichia coli (fluorescence)

The results for total coliforms, indicated by yellow wells, and Escherichia coli are as follows:

JULY 2024:

SAMPLES

DATE	Sample identification	Sample dilution	TOTAL coliforms (yellow appearance)			Escherichia coli (fluorescence)		
			Positive wells	MPN	TOTAL COLIFORMS MPN/100	POSITIVE WELLS	MPN	Escherichia coli MPN/100ML
2/7/24	5855/1	-	17	20.7	21	0	<1	<1
3/7/24	5475/12		4	4.2	4	0	<1	<1
3/7/24	5485/14		1	1.0	1	0	<1	<1
3/7/24	5485/15		1	1.0	1	0	<1	<1

3/7/24	5883/1		10	11.1	11	0	<1	<1
3/7/24	5890/1		51	>200.5	>200	46	118.4	120
08/07/24	5498/10		6	6.4	6	0	<1	<1
08/07/24	6015/1		16	19.2	19	0	<1	<1
10/07/24	5503/4		25	34.4	34	0	<1	<1
12/07/24	6139/1		4	4.2	4	0	<1	<1
12/07/24	6129/1		3	3.1	3	0	<1	<1
12/07/24	6129/2		2	2.0	2	0	<1	<1
15/7/24	5528/12		6	6.4	6	0	<1	<1
16/07/24	6185/1		1	1.0	1	0	<1	<1
16/07/24	6185/2		7	7.5	8	0	<1	<1
16/07/24	6185/4		4	4.2	4	0	<1	<1
17/07/24	5537/13		2	2.0	2	0	<1	<1
18/07/24	5587/1	10-1	43	94.5	94X10-1	4	4.2	4X10-1
23/07/24	5549/6		1	1.0	1	0	<1	<1
24/07/24	6335/2		1	1.0	1	0	<1	<1
24/07/24	6335/4		2	2.0	2	0	<1	<1

24/07/24	6335/5		5	5.3	5	0	<1	<1
24/07/24	BIOBALL		19	23.8	24	0	<1	<1
25/07/24	6377/2		5	5.3	5	0	<1	<1
25/07/24	5546/4		1	1.0	1	0	<1	<1
26/07/24	5568/14		3	3.1	3	0	<1	<1
26/07/24	6399/8		28	40.6	41	0	<1	<1
26/07/24	6399/9		25	34.4	34	0	<1	<1
29/07/24	6541/1		2	2.0	2	0	<1	<1
30/07/24	5606/10		1	1.0	1	0	<1	<1

CONTROLS

DATE	Sample identification	Sample dilution	TOTAL coliforms (yellow appearance)			Escherichia coli (fluorescence)		
			Positive wells	MPN	TOTAL COLIFORMS MPN/100	POSITIVE WELLS	MPN	Escherichia coli MPN/100ML
1/7/24	Ec13 10 ⁻⁸ +5ML Kpa10 ⁻⁸	-	47	129.8	130	42	88.5	88
2/7/24	Ec13 10 ⁻⁸ +5ML Kpa10 ⁻⁸		48	144.5	140	46	118.4	120

03/07/24	Ec13 10^- 8+5ML Kpa10^-8		49	165.2	170	38	69.7	70
04/07/24	Ec13 10^- 8+5ML Kpa10^-8		49	165.2	170	38	69.7	70
05/7/24	Ec13 10^- 8+5ML Kpa10^-8		45	109.1	110	37	65.9	66
08/7/24	Ec13 10^- 8+5ML Kpa10^-8		46	118.4	120	33	53.1	53
09/7/24	Ec13 10^- 8+5ML Kpa10^-8		43	94.5	94	31	47.8	48
10/07/24	Ec13 10^- 8+5ML Kpa10^-8		47	129.8	130	37	65.9	66
11/07/24	Ec13 10^- 8+5ML Kpa10^-8		46	118.4	120	42	88.5	88
12/07/24	Ec13 10^- 8+5ML Kpa10^-8		49	165.2	170	37	65.9	66
15/07/24	Ec13 10^- 8+5ML Kpa10^-8		44	101.3	100	36	62.4	62
16/07/24	Ec13 10^- 8+5ML Kpa10^-8		35	59.1	59	0	<1	<1

17/07/24	Ec13 10^- 8+5ML Kpa10^-8		44	101.3	100	37	65.9	66
18/07/24	Ec13 10^- 8+5ML Kpa10^-8		49	165.2	165	40	78.2	78
19/07/24	Ec13 10^- 8+5ML Kpa10^-8		43	94.5	94	34	56.0	56
22/07/24	Ec13 10^- 8+5ML Kpa10^-8		48	144.5	140	39	73.8	74
23/07/24	Ec13 10^- 8+5ML Kpa10^-8		43	94.5	94	35	59.1	59
24/07/24	Ec13 10^- 8+5ML Kpa10^-8		49	165.2	170	42	88.5	88
25/07/24	Ec13 10^- 8+5ML Kpa10^-8		45	109.1	110	37	73.8	74
26/07/24	Ec13 10^- 8+5ML Kpa10^-8		46	118.4	120	36	62.4	62
29/07/24	Ec13 10^- 8+5ML Kpa10^-8		44	101.3	100	40	78.2	78
30/07/24	Ec13 10^- 8+5ML Kpa10^-8		46	118.4	120	39	73.8	74

31/07/24	Ec13 10^-8+5ML Kpa10^-8		45	109.1	110	38	69.7	70
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On 16/07/24 positive control there were wrong results due to error in control sample preparation.

August 2024

DATE	Sample identification	Sample dilution	TOTAL coliforms (yellow appearance)			Escherichia coli (fluorescence)		
			Positive wells	MPN	TOTAL COLIFORMS MPN/100	POSITIVE WELLS	MPN	Escherichia coli MPN/100ML
2/8/24	6688/2	-	50	200.5	200	0	<1	<1
5/8/24	6410/4		7	7.5	8	0	<1	<1
6/8/24	6805/1		1	1.0	1	0	<1	<1
6/8/24	6805/2		8	8.7	9	0	<1	<1
6/8/24	6423/1		1	1.1	1	0	<1	<1
6/8/24	6822/1		51	>200.5	>200	51	>200.5	>200
12/08//24	6603/6		1	1	1	0	<1	<1
12/08/24	6607/12		27	38.4	38	0	<1	<1
13/08/24	6608/7		2	2.0	2	0	<1	<1

13/08/24	6923/2		1	1.0	1	0	<1	<1
15/08/24	6602/19		51	>200.5	200	0	<1	<1
15/08/24	6610/12		14	16.4	16	0	<1	<1
15/08/24	6938/1		33	53.1	53	0	<1	<1
16/08/24	6963/1	10 ⁻¹	46	118.4X10 ⁻¹	118.4X10 ⁻¹	5	5.3X10 ⁻¹	5.3X10 ⁻¹
16/08/24	6963/2	10 ⁻¹	49	165.2X10 ⁻¹	165.2X10 ⁻¹	3	3.1X10 ⁻¹	3.1X10 ⁻¹
16/08/24	6963/3	10 ⁻¹	51	>200.5X10 ⁻¹	>200.5X10 ⁻¹	1	1.0X10 ⁻¹	1.0X10 ⁻¹
16/08/24	6616/13		1	1.0	0	0	<1	<1
18/07/24	5587/1	10 ⁻¹	43	94.5	94X10 ⁻¹	4	4.2	4X10 ⁻¹
23/07/24	5549/6		1	1.0	1	0	<1	<1
24/07/24	6335/2		1	1.0	1	0	<1	<1
24/07/24	6335/4		2	2.0	2	0	<1	<1
24/07/24	6335/5		5	5.3	5	0	<1	<1
24/07/24	BIOBALL		19	23.8	24	0	<1	<1
25/07/24	6377/2		5	5.3	5	0	<1	<1
25/07/24	5546/4		1	1.0	1	0	<1	<1
26/07/24	5568/14		3	3.1	3	0	<1	<1

26/07/24	6399/8		28	40.6	41	0	<1	<1
26/07/24	6399/9		25	34.4	34	0	<1	<1
29/07/24	6541/1		2	2.0	2	0	<1	<1
30/07/24	5606/10		1	1.0	1	0	<1	<1

Results were consistent with sample 6822/1 showing significant levels of coliform and *E.coli*

Controls

DATE	Sample identification	Sample dilution	TOTAL coliforms (yellow appearance)			Escherichia coli (fluorescence)		
			Positive wells	MPN	TOTAL COLIFORMS MPN/100	POSITIVE WELLS	MPN	Escherichia coli MPN/100ML
01/08/24	Ec13 10 ⁻⁸ 8+5ML Kpa10 ⁻⁸	-	48	144.5	150	39	73.8	74
02/08/24	Ec13 10 ⁻⁸ 8+5ML Kpa10 ⁻⁸		49	165.2	170	41	83.1	83
05/08/24	Ec13 10 ⁻⁸ 8+5ML Kpa10 ⁻⁸		51	>200.5	>200	40	78.2	78
06/08/24	Ec13 10 ⁻⁸ 8+5ML Kpa10 ⁻⁸		47	129.8	130	40	78.2	78

07/08/24	Ec13 10^- 8+5ML Kpa10^-8		50	200.5	200	43	94.5	94
08/08/24	Ec13 10^- 8+5ML Kpa10^-8		49	165.2	170	40	78.2	78
12/08/24	Ec13 10^- 8+5ML Kpa10^-8		48	144.5	140	35	59.1	59
13/08/24	Ec13 10^- 8+5ML Kpa10^-8		43	94.5	94	36	62.4	62
14/08/24	Ec13 10^- 8+5ML Kpa10^-8		42	88.5	88	35	59.1	59
15/08/24	Ec13 10^- 8+5ML Kpa10^-8		46	118.4	120	40	78.2	78
16/08/24	Ec13 10^- 8+5ML Kpa10^-8		43	94.5	94	37	65.9	66
19/08/24	Ec13 10^- 8+5ML Kpa10^-8		45	109.1	110	31	47.8	48
20/08/24	Ec13 10^- 8+5ML Kpa10^-8		43	94.5	94	34	56.0	56
21/08/24	Ec13 10^- 8+5ML Kpa10^-8		45	109.1	110	30	43.3	45

22/08/24	Ec13 10^-8+5ML Kpa10^-8		51	200.5	200	35	59.1	59
23/08/24	Ec13 10^-8+5ML Kpa10^-8		45	109.1	110	32	50.4	50
29/08/24	Ec13 10^-8+5ML Kpa10^-8		42	88.5	88	38	69.7	70
30/08/24	Ec13 10^-8+5ML Kpa10^-8		44	101.3	100	34	56	56

Results were consistent throughout the period

SEPTEMBER

SAMPLES

DATE	Sample identification	Sample dilution	TOTAL coliforms (yellow appearance)			Escherichia coli (fluorescence)		
			Positive wells	MPN	TOTAL COLIFORMS MPN/100	POSITIVE WELLS	MPN	Escherichia coli MPN/100ML
3/9/24	7206/4	-	7	7.5	8	0	<1	<1
3/9/24	7491/11		26	36.4	36	0	<1	<1
3/9/24	7491/12		8	8.7	9	0	<1	<1
3/9/24	7504/1		3	3.1	3	0	<1	<1

3/9/24	7504/3		27	38.4	38	0	<1	<1
3/9/24	7504/4		2	2.0	2	0	<1	<1
5/09//24	7535/1		1	1.0	1	0	<1	<1
6/09/24	7587/1		1	1.0	1	0	<1	<1
9/09/24	7320/8		1	1.0	1	0	<1	<1
10/09/24	7664/1		19	23.8	24	0	<1	<1
10/09/24	7668/5		1	1.0	1	0	<1	<1
10/09/24	7668/8		11	12.4	12	0	<1	<1
11/09/24	7313/6		7	7.5	8	0	<1	<1
12/09/24	6963/2		4	4.2	4	0	<1	<1
16/09/24	7780/2		15	17.6	18	0	<1	<1
16/09/24	7359/6		2	2.0	2	0	<1	<1
16/09/24	7358/5		2	2.0	2	0	<1	<1
16/09/24	7358/8		7	7.5	8	0	<1	<1
16/09/24	7358/14		1	1.0	1	0	<1	<1
20/09/24	7377/1		51	>200.5	200	8	8.7	9
20/09/24	7938/2		36	62.4	62	0	<1	<1

23/09/24	7970/1		47	129.8	130	0	4	4
23/09/24	7725/9		8	8.7	9	0	4	4
27/09/24	Bioball		25	34.4	34	25	>4.4	34

Notable levels of coliforms on sample 7377/1 at 200 MPN/100ml and 9MPN/100ml *E.coli*

Controls

DATE	Sample identification	Sample dilution	TOTAL coliforms (yellow appearance)			Escherichia coli (fluorescence)		
			Positive wells	MPN	TOTAL COLIFORMS MPN/100	POSITIVE WELLS	MPN	Escherichia coli MPN/100ML
2/9/24	Ec13 10 ⁻⁸ +5ML Kpa10 ⁻⁸	-	48	144.5	140	38	69.7	70
3/9/24	Ec13 10 ⁻⁸ +5ML Kpa10 ⁻⁸		46	118.4	120	44	101.3	100
04/9/24	Ec13 10 ⁻⁸ +5ML Kpa10 ⁻⁸		47	129.8	130	43	94.5	94
05/09/24	Ec13 10 ⁻⁸ +5ML Kpa10 ⁻⁸		45	109.1	110	40	78.2	78

06/9/24	Ec13 10^- 8+5ML Kpa10^-8		49	165.2	160	39	73.8	74
09/9/24	Ec13 10^- 8+5ML Kpa10^-8		49	165.2	170	42	88.5	88
10/9/24	Ec13 10^- 8+5ML Kpa10^-8		48	144.5	140	40	78.2	78
11/09/24	Ec13 10^- 8+5ML Kpa10^-8		49	165.2	160	36	62.4	62
12/09/24	Ec13 10^- 8+5ML Kpa10^-8		0	<1	<1	0	<1	<1
13/09/24	Ec13 10^- 8+5ML Kpa10^-8		51	>200.5	>200	48	144.5	140
16/09/24	Ec13 10^- 8+5ML Kpa10^-8		44	101.3	100	37	65.9	66
18/09/24	Ec13 10^- 8+5ML Kpa10^-8		44	101.3	100	35	59	59
19/09/24	Ec13 10^- 8+5ML Kpa10^-8		0	<1	<1	0	<1	<1
20/09/24	Ec13 10^- 8+5ML Kpa10^-8		48	144.5	140	41	83.1	83

23/09/24	Ec13 10 ⁻⁸ 8+5ML Kpa10 ⁻⁸		46	118.4	120	37	65.9	66
24/09/24	Ec13 10 ⁻⁸ 8+5ML Kpa10 ⁻⁸		41	83.1	83	29	42.9	43
25/09/24	Ec13 10 ⁻⁸ 8+5ML Kpa10 ⁻⁸		47	129.8	130	35	59.1	59
26/09/24	Ec13 10 ⁻⁸ 8+5ML Kpa10 ⁻⁸		45	109	110	32	50.4	50
27/09/24	Ec13 10 ⁻⁸ 8+5ML Kpa10 ⁻⁸		50	200.5	200	47	129.8	130

On date 12/09/24 and 19/09/24 , the positive controls did not produce accurate results ,this was due to an error in forgetting to add colliert in the control samples .

Challenges Encountered

Procedural Errors

An operational error occurred in which media was not added to control samples during the **Ec13 10⁻⁸** test on two occasions 12th and 19th September. This error resulted in inconclusive controls and required re-testing to confirm the accuracy of the results.

On 16/07/24 positive control, there were wrong results(indicated negative for an E.coli positive control test) due to error in control sample preparation.

Recommendations

Implementing Procedural Controls

SAS Laboratory should implement a step-by-step procedural checklist to prevent errors such as missing media in control samples. Ensuring that all critical steps are followed will improve result accuracy and reduce delays caused by procedural oversights.

6.3 Automation for Greater Efficiency

Automating certain steps, such as sample labeling and MPN calculations, could help reduce manual errors and improve the overall efficiency of the testing process at SAS Laboratory.

7. Conclusion

The Enzyme Substrate Coliform Test results obtained at SAS Laboratory showed some inconsistency in positive test results due to procedural errors. By improving procedural controls, sample handling, and introducing automation, SAS Laboratory can further enhance the reliability and accuracy of its microbial water testing processes.

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