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Operation of an Onsite Legionella Laboratory

Adrian Parris,
BOHS President 2025,26
Occupational Hygiene & Chemical Safety Manager,
Sellafield Ltd

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Content

- Overview of Sellafield
- Summary of Journey
  - Requirement For An Onsite Legionella Laboratory
  - Laboratory Capability
- Method Validation
- Proposed Control Regime

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Overview of Sellafield Ltd

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Sellafield Ltd Today…

Spent Fuel Management

We are creating a clean and safe environment for future generations

- Safe, secure sustainable, site stewardship
- Progress at pace
- Lifetime value for money

Special Nuclear Materials

Value Streams. .

Remediation

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Requirement For Onsite Laboratory

- *Liquid samples leaving site require radiological screening.*
- *This delays plating time to 4 days.*
- *Outside of sample stability time ISO 11731 - Water quality. Detection and enumeration of Legionella.*
- *Can lead to false negatives.*
- *Lab Commissioned in 2018.*
- *Focussed on developing rapid testing capability.*

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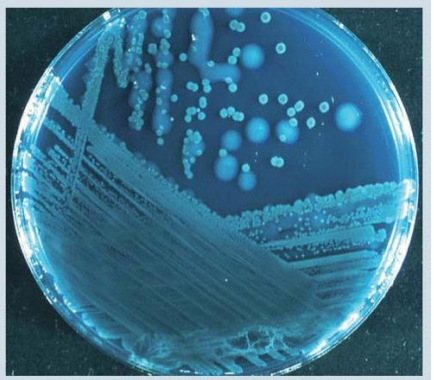
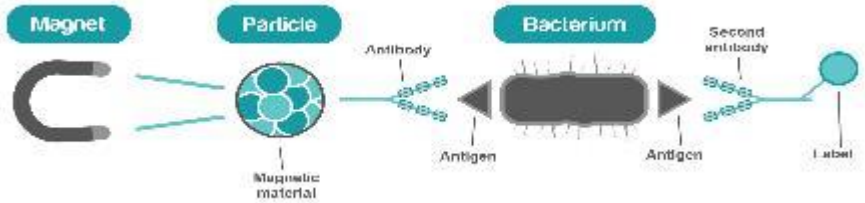
Onsite Microbiological Testing Capability

- *Testing Capability.*
  - *TVCs*
  - *Pseudomonas*
  - *Sulphate Reducing Bacteria*
  - *PCR*
  - *Legipid (IMS)*



- *Working towards UKAS accreditation.*
- *Routine monitoring and testing.*
- *Supported numerous projects.*
  - *Validation of TiAOP*
  - *Deadleg flushing*
  - *Biocide Efficacy Testing*



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Method Verification

- *Delay in completing.*
- *TVCs and Pseudomonas, good recovery, linearity & precision low LOD.*
- *PCR, Lower recovery rate than expected.*
  - *Firm ware update on Q16 thermocyclers.*
  - *Extraction from filter poor using orbital shaker.*
  - *Method revised to include bead beating.*
  - *Good recovery domestic and process water.*
- *Legipid*
  - *Low recovery rate.*
  - *Poor release of cells from filter.*
  - *Similar to recovery in culture method, 20 to 30%.*
  - *Method withdrawn.*

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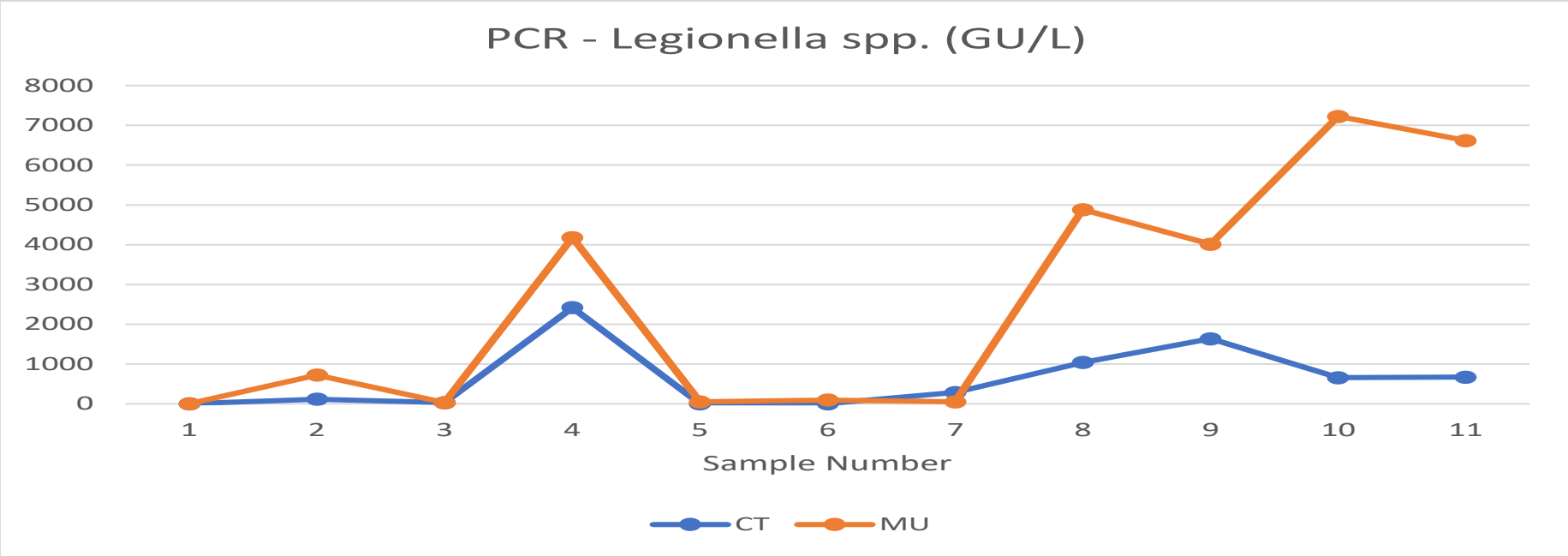
Control Levels Existing

	Date	Legionella Bacteria	Alert Level (GU/l)	Action Level (GU/l)
Lee et al.	2011	Pneumophila	1,000	10,000
		Species	100,000	1,000,000
Colins et al.	2017	Species (non differentiated)	1,000	10,000
Young et al.	2021	Species (non differentiated)	5,000	10,000

- *Still lack of data in the public domain.*
- *Only certainty is a negative PCR is accepted as negative.*

Proposed Control Regime – Cooling Towers



- How do we understand if PCR signal is showing system risk, differentiate from live or from dead legionella.
- Comparison of the level in the tower with the PCR signal from the makeup water key.
- Understanding if the system is supporting growth.

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Proposed Control Regime – Cooling Towers

	PCR (GU/l)		TVC's (cfu/ml)	Pseudomonas (cfu/ml)
	System	Makeup		
Incontrol	<5000			
Incontrol	Similar or lower than makeup	>5000	<1000	<1000

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Proposed Control Regime – Cooling Towers

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Proposed Control Regime – Cooling Towers

	PCR (GU/l)		TVC's (cfu/ml)	Pseudomonas (cfu/ml)
	System	Makeup		
Incontrol	<5000			
Incontrol	Similar or lower than makeup	>5000	<1000	<1000
Alert	>5000		>1000	>1000
Action	>10,000	<5,000 or <10% of tower GU in tower makeup i.e. a 1 log increase over makeup level		
Action			>10,000	>10,000

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Case Study 1
Marley Davenport Cooling Tower

Sample	PCR - Leg Species (GU/l)		%age Difference	TVCs (cfu/ml)	Pseuds (cfu/ml)	Control Status
	CT Return	CT MU	MU/CT	CT	CT	
1	28	20605	73589	10	10	
2	36	31976	88822	10	10	
3	1	78	7800	10	10	
4	9	1	11	10	10	
5	1	1	100	10	10	
6	148	12579	8499	20	30	
7	45	34678	77062	10	10	
8	33	12606	38200	10	10	
9	24	2243	9346	10	10	
10	4	524	13100	10	10	
11	95	8051	8475	10	10	
12	265	13011	4910	10	10	
13	366	2748	751	10	10	
14	1	15098	1509800	10	10	

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Case Study 1
Marley Davenport Cooling Tower

PCR - Leg Spec
CT Return
28
36
1
9
1
148
45
33
24
4
95
265
366
1

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Case Study 2 Watermiser Tower

Sample	PCR - Leg Species (GU/l)		%age Difference	TVCs (cfu/ml)	Pseuds (cfu/ml)	Control Status
	CT Return	CT MU	MU/CT	CT	CT	
1	1	3021	302100	90	40	
2	4882	24565	503	10	10	
3	1	1	100			
4	1	1	100	120	80	
5	6061	1	0	40	10	
6	25755	1	0	100	20	
7	71971	7177	10	110	30	
8	1	5979	597900	110	80	
9	11823	4261	36	90	80	
10	9217	1	0	70	10	
11	1	7324	732400	60	10	
12	6799	1	0	80	10	
13	4720	10480	222	40	40	
14	18190	11756	65	10	10	
15	1	4179	417900	20	10	
16	5016	4040	81	10	10	
17	6708	1557	23	50	20	
18	11520	1	0	480	10	
19	1	5820	582000	70	50	
20	443	7033	1588	30	10	

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Case Study 2 Watermiser Tower

Sample	PCR - Leg Species (GU/l)		%age Difference	TVCs (CFU/ml)	Pseuds (cfu/ml)	Control Status
	CT Return	CT MU	MU/CT	CT	CT	
17	6708	1557	23	50	20	

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Case Study 2 Watermiser Tower

Sample	PCR - Leg Species (GU/l)		%age Difference	TVCs (CFU/ml)	Pseuds (cfu/ml)	Control Status
	CT Return	CT MU	MU/CT	CT	CT	
14	18190	11756	65	10	10	

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Case Study 2 Watermiser Tower

Sample	PCR - Leg Species (GU/l)		%age Difference	TVCs (CFU/ml)	Pseuds (cfu/ml)	Control Status
	CT Return	CT MU	MU/CT	CT	CT	
18	11520	1	0	480	10	

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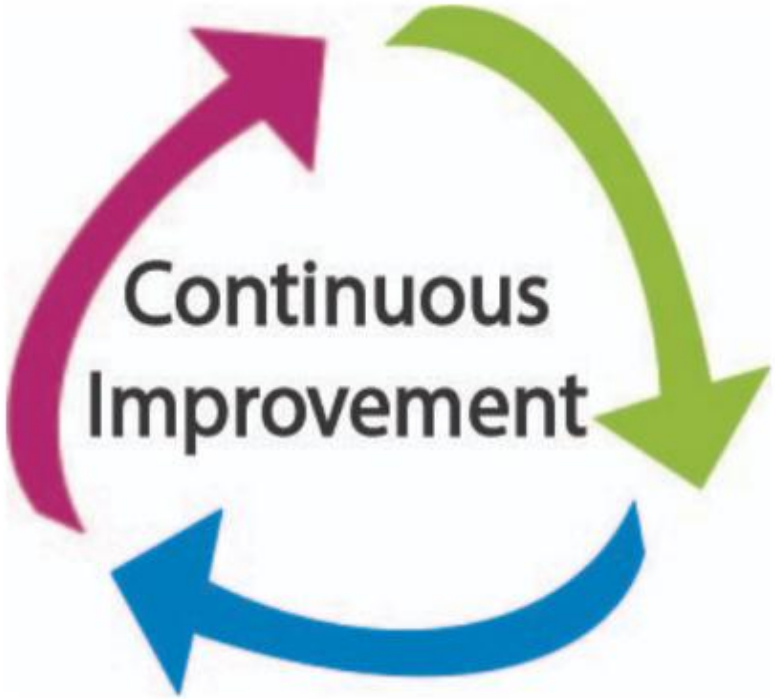
Key Steps

- *Endorsed by Duty Holders.*
- *Adopted into written schemes.*
- *Change in mindset.*
- *Continue exploring alternative analysis methods.*



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PhD – Research Rapid Detection Methods

- **BD flow cytometer** to detect total microbial load and intact microbial load within cooling tower samples.

- **Propidium monoazide (PMA),** a photo reactive DNA –binding dye used to selective detect viable microorganisms in combination with qPCR.

- **Genomadix cube** a viability PCR platform used to selective detect viable *Legionella pneumophila sg1*.

- **Digital Droplet PCR** a more precise method by determining the actual count of DNA molecules within a sample.

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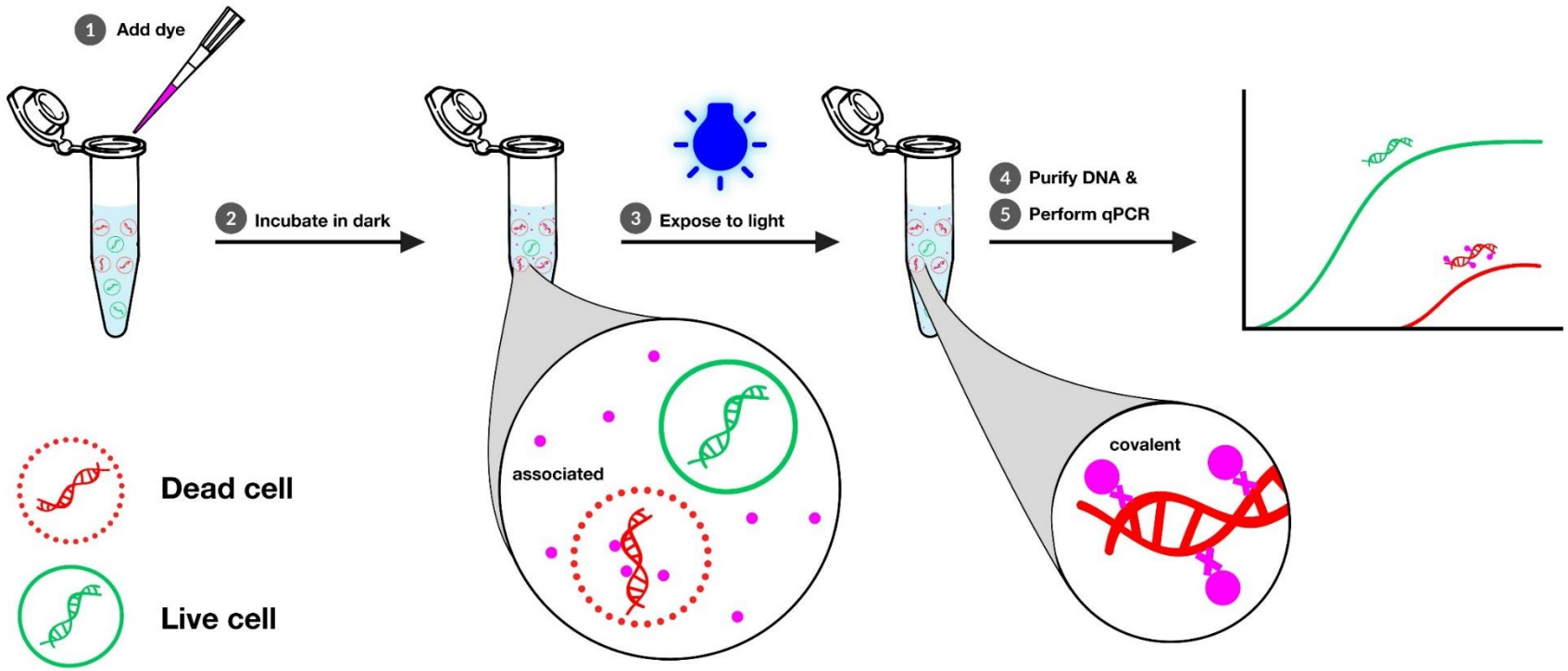
PhD – Research Rapid Detection Methods

- Propidium monoazide (PMA) is a photo reactive DNA –binding dye used to selective detect viable microorganisms by qPCR.



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Thank You to this Year's Exhibitors

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