Continuous Culture Based Monitoring for Qualitative Detection of Legionella Species Microbial Bioaerosols

Dr. Ruairi Monaghan[1], Roseanna Merriman[1], Prof. Stephen Daniels[1,2]

[1] NuWave Sensor Technology Ltd, DCU Alpha, Glasnevin, Dublin 9, Ireland

[2] Dublin City University, Glasnevin, Dublin 9, Ireland

Abstract

A new automated culture-based system for continuous, non-quantitative monitoring of the environment for airborne microbial contamination is presented. The system automates the air sampling process and uses a direct plating methodology, combined with a laser-based optical detection system, a local isolation and incubation environment, and a radio-based connectivity protocol for data analysis to provide an efficacious and widely applicable non-quantitative detection methodology. The results shown herein detail how a robust and efficient agar media was developed, able to minimise contamination from an array of contaminating bacteria present at live test sites, while retaining the ability to encourage the growth of legionella in a timescale comparable to laboratory incubation. The system is also shown to have a high collection efficiency of bacteria from the air at comparatively low environmental bioload levels, and that the adjustable system settings have an impact on the collection efficiency.

Keywords: automated microbiology; legionella detection; control system; biosensor

Introduction

Airborne microorganisms, or bioaerosols, are ubiquitous in the natural environment and an essential component of the ecosystem. However, certain microorganisms, such as legionella pneumophilia, can be problematic for human health when inhaled in aerosolised form, leading to disease. For example, it is known that aerosolised legionella pneumophilia can lead to the contraction of the life-threatening legionnaires disease (Cunha *et al.*, 2016) and consequently in most jurisdictions various standards and processes exist for routinely testing for the presence of legionella in likely habitats that could result in aerosolised legionella coming into contact with humans. In its natural habitat, in nature or in anthropogenic ecosystems such as potable water systems, legionella is a ubiquitous aquatic pathogen that is able to produce a monospecies biofilm, but is also able to survive in multispecies biofilms (Abdel-Nour *et al.,* 2013). The bio-aerosolization process for legionella remains poorly understood and is likely to be multifactorial, however the droplet formation can result from mechanical processes in water fountains, shower heads etc. The aerodynamic diameter of the droplets can impact the viability and pathogenicity of the legionella bioaerosol (Allegra *et al.*, 2016) as well at the dynamics of the dispersion in the environment. This is further confounded by the fact that the lifetime of a water droplet in the air is determined by evaporation and fracture by dynamic stresses, coalescence with other droplets and growth through condensation (Bourouiba and Bush, 2013).

Culture based methods for detecting microorganisms are long established and can be applied to both quantitative and qualitative testing (Abbas *et al.*, 2004). This method employs nutrient media that facilitates and encourages the growth of the target organism which in turn results in a change in the property of the medium, such as colour, turbidity, observation of colonies, etc. The preparation, storage and performance of the agar medium is critically

important and various standards exist relating to these quality constraints, including for example ISO 11133, which details a standard for the preparation, production, storage & performance testing of media for investigating the microbiology of food, animal feeding stuffs, and water (International Organisation for Standards [ISO], 2014).

Here we introduce a new method for the detection of airborne microorganisms based on the traditional culture method, but with automated, continuous sampling and an optical, laser-based system, for detection of attribute changes in an agar delivered by the selective growth of a target organism. The device, termed LumAir^{TM}, was developed by NuWave Sensors and the engineering details are described in US Patent No, 10,456,785.

Automated sampling technique

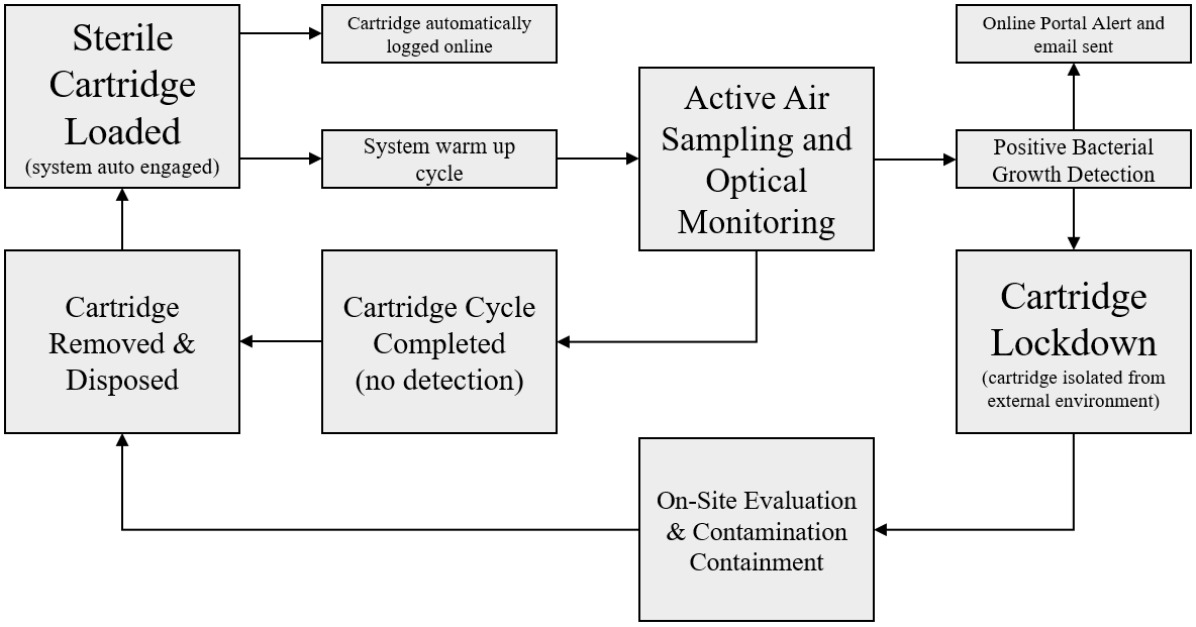

Fig. 1. Block Diagram Detailing the Use of Cartridges in the System

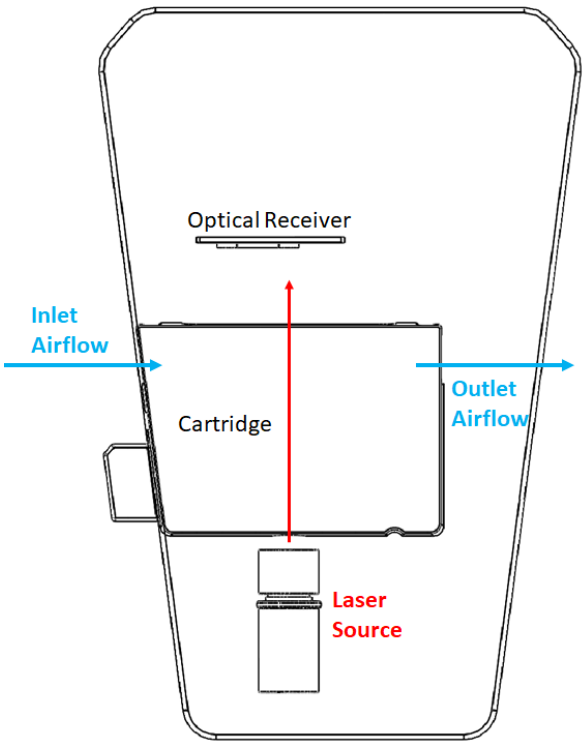

Fig. 2. LumAir™ system line drawing, showing the positioning of the laser system relative to the cartridge, and the direction of air flow during sampling

The LumAir™ automated system works in conjunction with an agar-based media component housed in a cartridge. The use of the system and cartridge is given in figure 1, and displayed visually in figure 2. Here we show that once loaded, the system begins sampling air through the inlet of the cartridge. Air will be cycled periodically though the cartridge for up to 2-weeks, and during this time a laser based optical monitoring system will monitor for growth of target organisms in the cartridge. The primary agar for detection, which contains nutrients, supplements, inhibitors, and other additives, is contained within this cartridge arrangement as illustrated in figure 3, which is supplied by NuWave Sensors. The mode of microbial isolation of the cartridge is also illustrated in figure 3. During operation the air is periodically pulled into the cartridge interior and circulated over the agar surface to encourage particle deposition. Once an air exchange has occurred, the cartridge is isolated from the external environment via cut-off paddles (shown in purple). As the agar media section of the cartridge is incubated

throughout, this creates small convection currents with the air in the exchange section, further encouraging the suspended particles to migrate to the agar segment of the cartridge, where it can settle on the surface. As can be seen in figure 3, the agar is housed in a deep, tailored transparent “vessel” which, when in active use, is placed into the habitat-controlled sampling device. Prior to installation the agar and cartridges are prepared in a sterile environment, and contained in sterilized packaging. All cartridges are manufactured sterile, and during system operation air is only ever drawn through the cartridge, ensuring no cross-contamination between the system and the cartridge is possible. Guidance relating to the maximum storage times for prepared media is given in ISO 8199 and in work by Blodgett, and this is of course a significant challenge for the new technique discussed here, as it is necessary for the media to be in use for an extended period, for example up to two weeks if conditions and the application allows (ISO, 2005; Blodgett, 2002). During this time no colour change, significant evaporation/dehydration, change in pH, or severe modification of the additives is acceptable as this may impact the efficacy of the media (Katz and Hammel, 1987). Note that when the system is activated, the media is maintained in constant darkness except for when being probed by the laser.

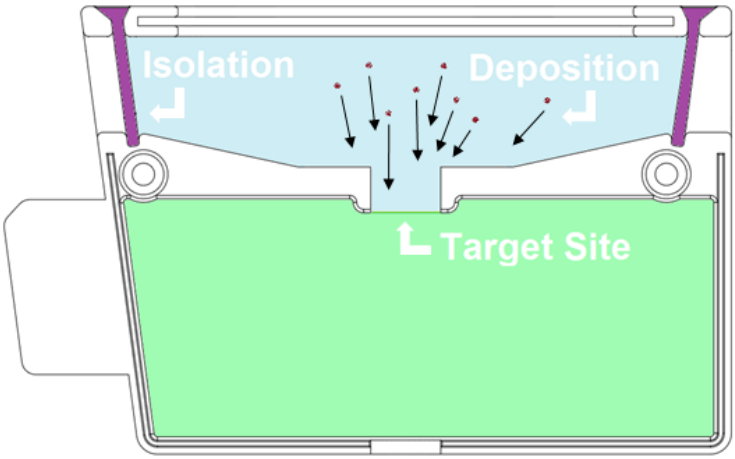

Fig. 3. Cartridge Illustration for Automated Detection System Showing Isolating Shutter System to Isolate the Cartridges Internals from the External Environment

The laser and optical system also contain a number of redundancies and self-calibrating capabilities to deal with instances of sharp signal changes. For instance, in the event that a large particle of dust or an insect partially blocks the laser trajectory, the system adjusts for the subsequent step function in signal intensity, and re-calibrates to continue monitoring. If the occlusion is too large for normal operation to continue, a warning is sent out via the online system, and the cartridge may need to be replaced.

There is a risk when exposing settle plates in high airflow environments, such as laminar airflow cabinets, microbiology safety cabinets, and impactors, that there is significant desiccation of the agar during exposure, which is why exposure is typically capped at four hours for conventional settle-plate sampling. Unfortunately, this is difficult to avoid if the agar is directly interacting with substantial airflow. To deal with this, the method described here removes the surface of the agar from direct interaction with moving air, instead creating a circulating air volume above the agar, propagated by internal air convection currents due to the onboard heating. This is engineered to encourage solid and liquid matter such as particulates, droplets, etc to settle on the agar which is located in a lower chamber, away from horizontal airflow, whilst minimising desiccation and other defect modes. A useful, albeit incomplete, indicator of the state of the agar is the weight-loss and its effect on the growth efficacy (Sandle, 2015). Over a 2-week period, typically, the managed agar in the cartridge experiences a weight loss approximately equivalent to a 4 hour settle plate and the recovery efficiency is also approximately the same. In the automated process the sampling and incubation process run concurrently. It is also worth noting that long term incubation (e.g. >2 weeks) in a humid atmosphere prevents major desiccation of the agar and this is mimicked in the controlled habitat of the automated system.

To assess the level of moisture loss (loss of mass) of the agar, a set of experiments were performed on the manufactured agar cartridges. The data is shown below in table 1.

Table 1. Moisture Loss in Cartridges in Relation to External Sampling Humidity

Experiment Set	Humidity Range (% rh)	Water Loss (%)
1a	*31-49*	*24.4*
1b	*31-49*	*25*
1c	*31-49*	*24*
2a	*85-99*	*15.5*
2b	*85-99*	*14.4*
2c	*85-99*	*14.4*

As can be seen, the environmental humidity plays a large role in the rate of water loss of the agar, as expected. Cartridge isolation during sampling periods helps to delay any excess moisture loss, but any exposure to air will leech water from the agar bulk in general. As temperature is maintained at a set value in the cartridge, the environmental temperature is not considered a contributing factor. To assess the viability of the agar, post a two-week cycle, a set of experiments were performed to attain information relating to the water activity (a_w) of the agar, an important factor for the growth of bacteria. It is known that a_w has a greater effect on the ability for bacteria to propagate and thrive than just water content of a substance. By definition, a_w is the ratio between the water vapour pressure of the substance and the surrounding air, compared to pure water in identical conditions. Although affected over time by the water content of the substance, they are not linearly related. The results of the a_w experiments are shown in table 2. In this experiment, several sets of samples were left out for varying timescales, and the a_w being tested immediately after.

Table 2. Water Activity as a Function of Agar Moisture Loss

Experiment Set	Moisture Loss (%)	Water Activity (a_w)
Control	*0.0*	*0.992*
1 Hour	*17.32*	*0.987*
2 Hours	*28.33*	*0.988*
3 Hours	*39.66*	*0.983*
4 Hours	*54.37*	*0.963*

As is clearly shown, drying of the agar, within the limits allowable for our arrangement, still yields high levels of a_w. All bacteria have different preferred a_w levels for growth, but in general the higher the a_w the better. Many bacteria will become unable to propagate if the a_w drops below 0.9, and for some above 0.97 is required (Beuchat, 1981; Barbosa-Cánovas, 2008). Although this is only one factor that can affect bacterial growth on an agar (deformation/cracks, nutrient ratio shift etc) it is an important one, and shows a perfectly balanced agar can survive a full two-week cycle and retain essential characteristics that promote good bacterial growth

The environmental sampling method and the process of introduction of the sample onto the agar is critically important. In the case of air sampling for legionella, the air transport mechanism is in water droplets, and the volume of fluid/water introduced onto the agar media depends on environmental conditions such as relative humidity, dew point, temperature, and the air sampling regime. Furthermore, as the aerosols are transported across the agar, or more correctly the cavity in which the agar is contained, the efficiency of the deposition of the particulates onto the agar needs to be considered. In the method described here, the air is circulated in the habitat and the particulates are held long enough to encourage them to settle. In this case the individual sample volume is small and the limit of detection is best calculated over a 24-hour period.

As the water droplet size in an active system (such as a water tower) can be large, especially in comparison to the size of the microbe in question, the droplets can be assumed

to act in a similar way to particles. In the case of water towers, droplets may range from 0.05 – 0.08mm in diameter, and possibly larger. In calculating the number of viable particles interacting with the agar, the key variables include: the minimum number of viable particles/m3 in the air, the sheer stress coefficient that determines the mortality attributed to the mechanical interactions of the microorganisms and the agar, the flow rate through the device in l/min, the sampling device duty cycle and the viable particle deposition efficiency.

The system limit of detection is also determined by the limits of the optics of the detection system, which is subsequently determined by the earliest stage of development of a bacterial colony capable of causing an optical interference event. The collection efficiency shown in Figure 4 relates the resolution of the optical system, the number of viable particles in the sampling volume, and the overall efficiency of viable particle deposition onto the agar in the optical detection system.

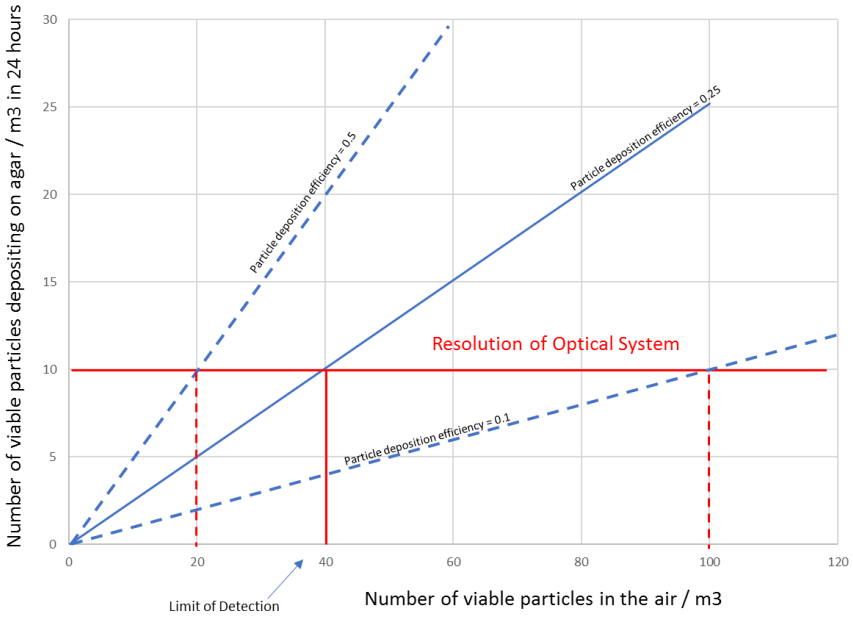

Fig. 4. LumAir^{TM} Limit of Detection

The LumAir^{TM} system is fully automated and designed to be located remotely at the most efficacious test point for airborne testing. The device has an on-board radio for communicating to a cloud-based server for remote real-time data monitoring and analysis. There are multiple advantages to this method of air sampling. First, specific bacterial colonies incubated at an appropriate temperature typically grow at a relatively predictable rate, allowing for extrapolation of when the infection occurred. Coupling the timing of the detection, with historical temperature and humidity data, infection occurrences can be timed with good accuracy. Secondly, the constant operation of the unit allows for good 'outbreak' detection. Sampling of airborne microbes is often performed on a bi-weekly, potentially monthly cycle. A lot can happen within that time, and a significant increase in bacterial load could occur during this window without the knowledge of the user. Having a constant monitoring point alleviates some of this risk. And thirdly, information gained on timed detection of pathogens can influence the cleaning/testing practices of environments.

Legionella Detection Process

Legionella can pose a serious health risk and consequently effective procedures for monitoring and controlling legionella are essential. ISO 11731 2nd Edition is an international standard detailing culture method for the isolation of Legionella and estimation of their numbers in water samples (ISO, 2017). The translation from water sample concentrations to airborne droplet bacterial concentrations is still a matter for debate but there is no doubt that airborne legionella is dangerous, including posing a risk to public health in public spaces (Prussin *et al.*, 2017). There are 58 species of Legionella but not all species of Legionella are currently known to be pathogenic. Of them, approximately 24 are known to effect human health (Newton *et al.,* 2010). When a strain of Legionella is pathogenic, it may cause a

pneumonic disease called Legionellosis, and in a less severe form, Pontiac fever, which causes influenza-like symptoms. It is important to note that not all strains of legionella are culturable and hence not detectable by the methods discussed in this paper. For this work we will therefore be focussing on legionella pneumophilia, as it is one of the most pathogenic of the species.

In preparation for analysis, typically legionellae in a water sample are concentrated by membrane filtration, diluted or directly plated onto an agar media depending on the characteristics of the sample and the anticipated numbers of legionellae. In instances of legionella outbreaks, the number densities of legionella are expected to be high and in this case a concentration step may not be necessary. If the number densities of legionellae are expected to be high then the same applies to other non-target bacteria which can interfere with the recovery of legionellae. To minimise this interference, samples can be subjected to heat treatment and/or acid treatment. Table 3 is taken from ISO 11731 and indicates the advantages and disadvantages of the different sampling and plating methods (ISO, 2017).

Table 3. Plating Methods Matrix

Method	Advantages	Disadvantages
Direct Plating	*Easy to count* *Good recovery*	*High limit of detection*
Membrane Filter on Plate	*Easy method* *Low limit of detection*	*Difficult to count (due to overgrowth of interfering microorganisms)* *Influence of the membrane filter*
Filtration with Washing Procedure	*Easy to count* *Low limit of detection*	*Lower recovery (compared with direct plating method and membrane filter on plate method)* *Time-consuming*
Plating after Dilution	*Easy to count* *Good recovery*	*High limit of detection* *Time-consuming*

ISO 11731 also gives guidance as to the most appropriate sampling method to use based on the origin and characteristics of the sample (ISO, 2017). The direct plating method is most appropriate in cases where there is an expected low concentration of interfering microorganisms, e.g. potable water and an expected high concentration of Legionella species, circa > 10^4 cfu/l. The automated sampling method described above is a direct plating process as there is no mechanical filtering, washing, or dilution employed.

If the concentration of interfering microorganisms is high, the ISO 11731standard recommends heat and/or acid treatment. For legionella it is recommended that a small volume sample (≤ 5 ml) is maintained at (50 ± 1) °C for (30 ± 2) min, with more detail provided for several samples and large volume samples. The heat treatment is essentially a decontamination process with a proportion of the non-target microorganisms rendered not-viable due to the heat exposure. This process can be approximated, but is difficult to replicate precisely in the automated method as rather than a single sample volume that can be heat-treated, multiple much smaller samples are introduced on a continuous basis. However, periodic elevations in temperature can be useful, which in essence is an integrated incubation and heat-treatment process. During the integrated process it is important that the legionellae is not allowed to dry out as this can affect its viability.

The measurement method described earlier requires a laser to pass through the volume of agar contained within a vessel in the cartridge and the target organism, if present, is cultured on the media surface and interacts with the laser, resulting in absorption and scattering of the light signal which is subsequently detected by an array of optical detectors. A transparent agar is desirable to facilitate this.

Several media have been synthesised for the cultivation and isolation of legionella species for the past several decades, with Buffered Charcoal Yeast Extract (BCYE) agar being the most commonly used media due to its efficacy in supporting the growth of a range of

legionella species. Due to the presence of charcoal, this agar is dark, or opaque, and this is unsuitable for use with the optical system described herein. For a number of performance related reasons, a number of clear agar formulations have been developed. These include a Yeast Extract-Phosphate-Hemin (YPH) medium, which does not contain charcoal but has efficacies comparable with that of the BCYE agars, as well as Legionella Transparent Medium (LTM) media, described by Armon and Payment, which again foregoes the use of charcoal as an ingredient and shows excellent recovery and selectivity when supplemented with antibiotics (Armon and Payment, 1990).

Experimental – Bioaerosols/General Bacteria

To assess the systems' ability to capture and grow bacteria in a low bacterial load (relative to levels described in the Commission of European Communities. Biological particles in indoor environments) environment (100-500 CFU/m^3), a set of experiments were performed using multiple LumAir^{TM} systems to investigate the collection efficiency of bacteria. Herein a general-purpose TSA agar cartridge was employed (NuWave Sensors LC-NS). The results for a full two-week cycle are shown in Table 4 (data gathered from the environment using TSA agar with a Sampl'air Lite impactor, with several samples taken daily for the 2-week period), alongside the subsequent set of accompanying pickup rates of the LumAir^{TM} system at four specific sampling settings.

Table 4. Air Sampling using varying settings in a work environment

	Environmental CFU/m3		Efficiency (Pickup)	Earliest time to Detection (hrs)	Humidity Range (rh %)	Temperature Range (Celsius)
Average	202.8	**Experiment #1 (Settings A)**	66%	66	45 - 57	21 - 25
Max	289.7	**Experiment #2 (Settings B)**	100%	95	39 - 48	19 - 23
Min	125.5	**Experiment #3 (Settings C)**	100%	81	40 - 50	17 - 22
		Experiment #4 (Settings D)	50%	171	39 - 60	13 - 21

As can be seen, the collection efficiency is 100% for the two of the system settings, B and C, whereas setting A shows a 66% efficiency, and D a 50% efficiency. As expected, this is due to the system settings having an impact on the collection efficiency. By limiting the amount of time the system samples the air we lower the chances of collecting microbes in the air. Timing settings for air sampling etc are controlled via the cloud server, and can adapt in real time based on detected environmental factors and the targeted pathogen. For these experiments the timing settings were locked in from the beginning. A trade off of highly extended sampling times of course increased potential of agar desiccation, something that is affected by environmental factors also, i.e. humidity levels, so limits exist in the system logic to maintain the two-week cartridge lifetime in the field.

Release of bacteria species in an aerosolised form (nebulisation etc) was deemed too extreme, as the potential contamination of workspace and other experiments, and possible health implications, were considered too high.

Experimental – Legionella

Validation of the transparent legionella growth media was an integral step in development of the remote monitoring system. For this purpose, legionella pneumophilia was inoculated on multiple cartridges, and the subsequent growth was monitored using several automated units. Inoculations were carried out by dispensing 100 uL of legionella suspension onto the surface of each chosen cartridge. The suspension was consistently measured at 0.6 on the McFarland standard scale before inoculation during every experiment to allow for relatively comparative results between experiments. Plated controls alongside the cartridges showed growth of 50-75 colonies from similar 100 uL aliquots spread across the surface, showing the inoculation density for the cartridge to be high. The positive detections displayed in figure 5 were shown to have 3-5 colonies in the detection zone at the time of experiment end.

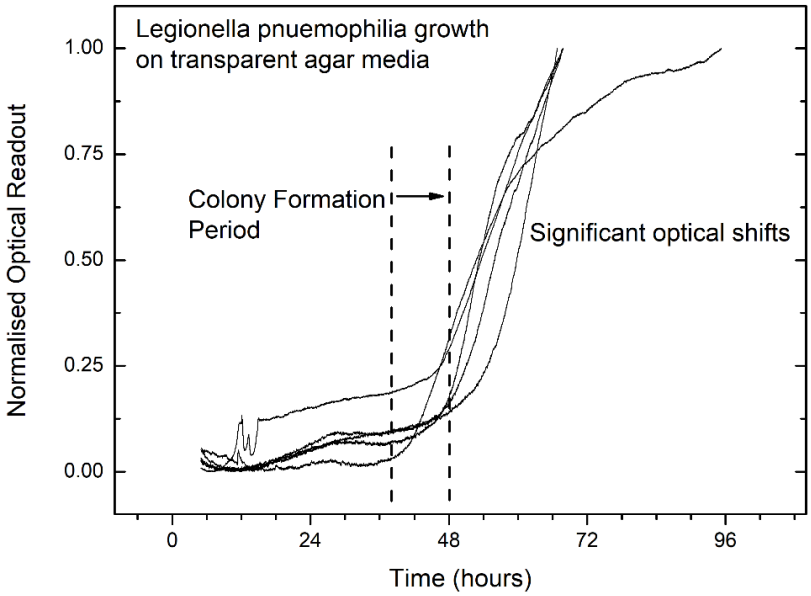

Fig. 5. Legionella growth detection employing clear agar in automated monitoring system, completed on 5 different automated LumAir^{TM} units in parallel

After an initial stabilising time of 5 hours, where incubation temperature was reached (36-36.5^{O}C), laser signal data was plotted and normalised for closer comparison. As shown in figure 5, as the time approaches the 2-day mark (48 hours) a significant shift in optical signal response could be seen, indicating the early stages of colony formation. Once the shift occurred, the rapid growth characteristic of bacterial colony formation continued until the experiment completed.

Cartridges (NuWave Product Code LC-LG) for this experiment were inoculated, and then immediately sealed using a clear lid and adhesive so that the bacterial strain could grow unhindered, and not risk contaminating the lab space or automated unit.

To ensure the signal detected was directly from legionella colonies, and not a possible contaminant strain of bacteria, the growths were analysed using MALDI-TOF MS (Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry). All results came back positive for legionella pneumophilia, with a score value range of 2.25 – 2.44, which is high enough to be a definitive analysis.

Facilitating legionella growth detection alone however is not sufficient as the sole function of the cartridges. Robust resistance to environmental contaminants, such as native and competing bacteria is also a requirement if the cartridges are to be used in the field. Positive and negative controls of all agar batches are performed to ensure legionella growth will be efficient, and also assess that sterility and resilience to infection is maintained at adequate levels. The four contaminating bacteria chosen for lab use are:

- Enterococcus faecalis (gram-positive)
- Escherichia coli (gram-negative)
- Staphylococcus epidermidis (gram-positive)
- Pseudomonas aeruginosa (gram-negative)

The reason for these bacteria to be chosen was to cover 2 sets of gram positive/negative bacteria, and show the resilience of the agar with respect to these. As the units are designed to be installed in a broad range of facilities/environments, it was deemed acceptable for a first-generation cartridge to show moderate bacterial resistance to other bacteria while still allowing legionella growth.

Several sets of experiments were conducted, with plates being divided into different segments, each being infected with an increasingly dense concentration of the contaminating bacteria. Results from these control tests are shown in the table 5.

Table 5. Legionella Cartridge Contamination Control Test Results

Inoculation	**Conc 10^2**	**Conc 10^3**	**Conc 10^4**	**Conc 10^5**
E. faecalis	*Inhibited*	*Inhibited*	*Partial inhibition*	*No inhibition*
E. coli	*Inhibited*	*Inhibited*	*Inhibited*	*n/a*
S. epidermidis	*Inhibited*	*Inhibited*	*Inhibited*	*n/a*
P. aeruginosa	*Inhibited*	*Inhibited*	*Inhibited*	*Inhibited*

As can be seen from table 5, the ability to inhibit the chosen bacteria is high. E. faecalis has proven more resilient than the others at high concentrations, but this concentration of any bacterial species should be rare as an aerosol in natural environments, and is considered acceptable here. The ability of the automated units to incubate the cartridges at specific temperatures (creating unfavourable environments for contaminants to grow) also strengthens the overall specificity of the technology.

A LumAir^{TM} unit was deployed during early development at a water tower facility, known to have high bacterial contaminant levels, to assess the in-field capabilities of the system. Housed in a weather proof box, and piped directly into the cooling towers main cavity, air was constantly drawn into the enclosure by a fan mounted on the outlet. Initial experimentation was conducted using a cartridge designed to pick up a wide range of microbes (agar media with antibiotics removed). This approach characterised the microbial diversity in

this environment, and what aerosolised contaminants were present in high quantities. Three different cycles were performed for this experiment. Each lasted only a short length of time before an obvious signal shift had occurred, as can be seen in figure 6.

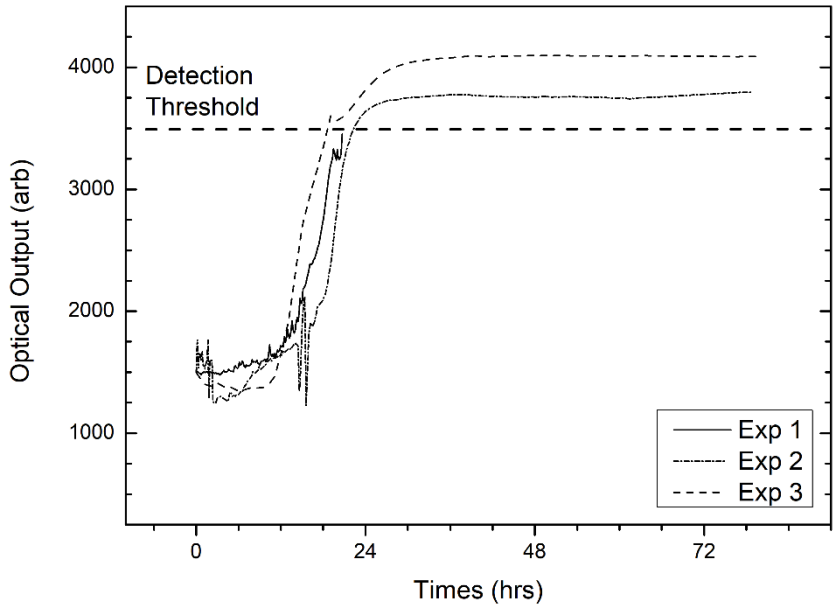

Fig. 6. Experiments performed at the external water tower facility, showing the rapid collection and propagation of contaminant bacterial species present in the water

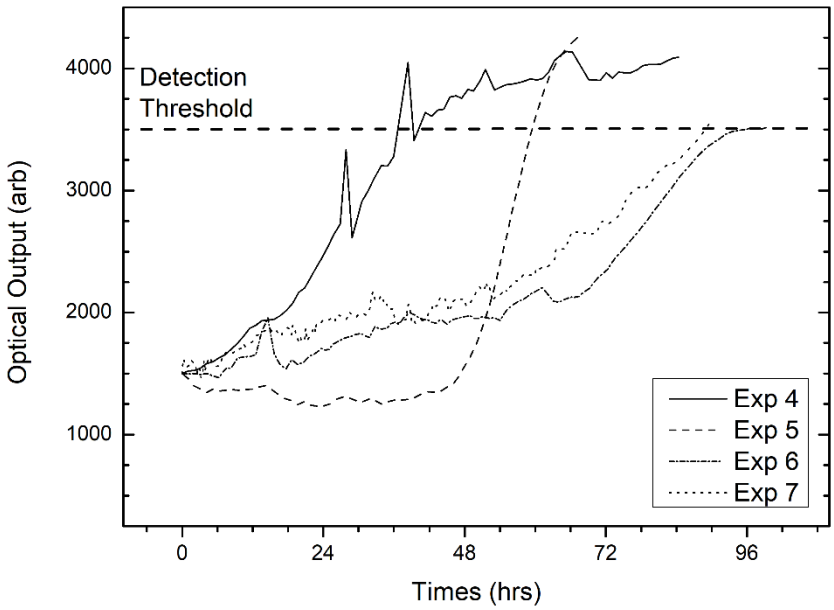

Fig. 7. Generation 1 Legionella Cartridges with Broadband Antibiotics

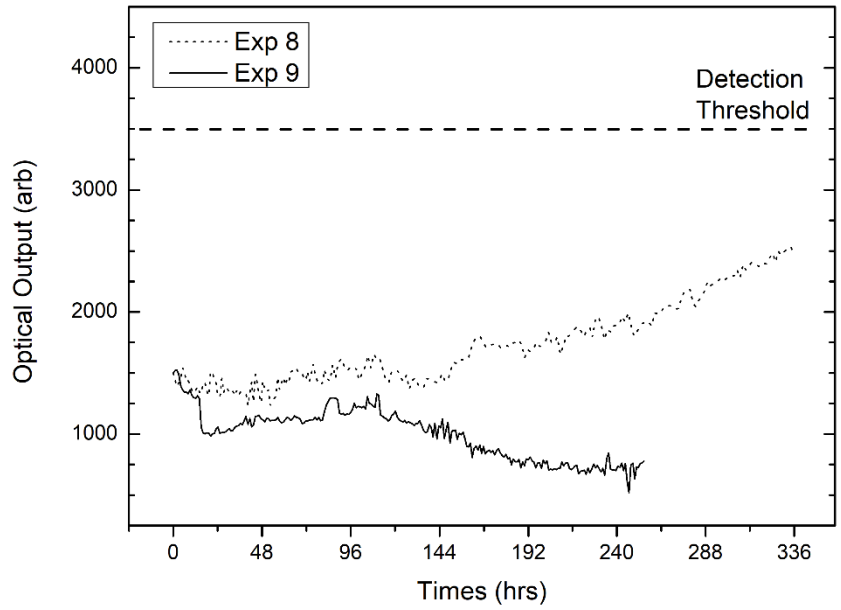

Fig. 8. Generation 2 Legionella Cartridge Robustness Against Contamination

A separate verification of the bacterial growth was carried out. It was determined that the air being drawn from the cooling tower was heavily contaminated with the following bacteria:

- *Aeromonas hydrophila (gram negative)*
- *Bacillus cereus (gram positive)*
- *Acinetobacter beijerinckii (gram negative)*
- *Acinetobacter junii (gram negative)*

During testing, the humidity range swung greatly, from 42% - 100% rh, while the temperature stayed within a range of 23-34 degrees Celsius. The large swing in humidity is related to the operation of the water tower itself, whereas the temperature is just seasonal based.

In response to these findings, another set of experiments were performed using first generation legionella specific cartridges, installed in the same unit. The cartridge media, with its combination of specific antibiotics, had shown high yields of legionella growth, and high

to moderate inhibition of the chosen contamination pathogens in a laboratory setting (as described previously). As can be seen from figure 7, the time-to-growth event for contaminant species was substantially extended, but the risk of contaminating the agar over a two-week period remained, albeit in a much weaker capacity.

Further analysis of the cartridges yielded a secondary set of contaminating bacteria, present in high enough quantities to eventually overcome the antibiotics already present in the first-generation cartridges. The contaminating bacteria were:

- *Alcaligenes faecalis (gram negative)*
- *Ralstonia pickettii (gram negative)*

During this second phase of testing again the humidity varied heavily, from 44% - 100% rh, and the temperature dropped to between 1-16 degrees Celsius as the weather became colder.

In response to these contamination events, a second generation of legionella-based cartridges was developed (the current LC-LG formulation), amending the combination of antibiotics to prevent contamination from a wider variety of bacterial growth, whilst maintaining the legionella recovery efficacy. Specifically in this case, the gram negative antibiotic ratio was shifted and brought higher to ensure stability over the two-week period. Figure 8 shows the outcome of these changes.

As can be seen on the plot in figure 8, the full 2-week cartridge cycles completed without incident. At the time of analysis, the water tower itself was subjected to routine water-based analysis (Phigenics Validation Test), and it was found that legionella was not present. The legionella-based cartridges have shown good resistance to a range of common bacterial and fungal contaminants present at the test site and in laboratory bench tests.

Conclusion

Automating the air sampling and agar culturing processes for remote, continuous monitoring of microbial contamination has been shown to be feasible and a very practical method of environmental monitoring. From the data presented for legionella, good recovery of the target microorganism and high specificity can be achieved assuming correct deployment and an understanding. New novel methods for effective detection of legionella are emerging, using for example microfluidic systems, desktop qPCR systems, and culture systems as described herein, and this is being driven by the need for better technologies to aid the detection and control of legionella in the environment (Yamaguchi, 2017; Spartanbio, website).

Combining the system with traditionally implemented verification testing methods, it becomes an effective augmentation to the current state-of-the-art monitoring practices in place currently. The addition of a continuous monitoring point can aid greatly in rapidly responding to bacterial outbreaks. It can also help in understanding the evolution of bacterial outbreaks in at risk areas, allowing for future implementation of more efficient preventative actions, providing a broader safety net for at risk sites that require tight management. Although highly specific at identification, through the combined optical and agar specificity, cartridges can be treated like standard collection plates and tested in a laboratory for definitive identification for any positive detections.

References

Abbas, H.K., Shier, W.T., Horn, B.W. and Weaver, M.A. (2004). Cultural Methods for Aflatoxin Detection. *Journal of Toxicology.* 23:295-315

Abdel-Nour, M., Duncan, C., Lowand, D.E. and Guyard, C. (2013). Biofilms: The Stronghold of Legionella pneumophila. *Int J Mol Sci.* 14(11): 21660-21675

Allegra, S., Leclerc, L., Massard, P.A., Girardot, F., Riffard, S. and Pourchez, J. (2016). Characterization of aerosols containing Legionella generated upon nebulization. *Scientific Reports.* 6: 33998

Armon, R. and Payment, P. (1990). A transparent medium for isolation of Legionella pneumophia from environmental water sources. *Journal of Microbiological Methods.* 11: 65-71

Barbosa-Cánovas, G.V., et al, (2007), *Water Activity in Foods: Fundamentals and Applications*, IFT Press, Washington

Beuchat, L.R. (1981). Microbial stability as affected by water activity. *Cereal Foods World.* 26: 345–349

Blodgett, R. (2002). Measuring improbability of outcomes from a serial dilution test. *Communications in Statistics: Theory and Methods*. 31(12): 2209–2223

Bourouiba, L. and Bush, J. W. M. (2013). Drops and bubbles in the environment. *Handbook of Environmental Fluid Dynamics.* Vol. 1, H.J.S. Fernando (Eds.), CRC Press, Taylor & Francis Book Inc

Cunha, B.A., Burillo, A. and Bouza, E. (2016). Legionnaires' disease. *The Lancet*. *387*: 376-385.

Daniels, S., Phelan, S. and Monaghan, R. US Patent Number 10,456,785. Method and apparatus for remote identification and monitoring of airborne microbial activity.

ISO 11133:2014, Microbiology of food, animal feed and water -- Preparation, production, storage and performance testing of culture media.

ISO 11731:2017, Water quality -- Enumeration of Legionella

ISO 17994, Water quality — Requirements for the comparison of the relative recovery of microorganisms by two quantitative methods

Katz, S.M. and Hammel, J.M. (1987). The Effect of Drying, Heat, and pH on the Survival of Legionella pneumophila. *Annals of Clinical and Laboratory Science.* 17:150-156

LumAir™ official product webpage, https://nuwavesensors.com/product/lumair-lu45, Last Access: 22 October 2019. **[Comment added February 2023, URL superceded by https://lum-air.com/products/]**

Newton, H.J., Ang, D.K., van Driel, I.R. and Hartland, E.L. (2010). Molecular pathogenesis of infections caused by Legionella pneumophila. *Clinical microbiology reviews*. *23*(2): 274-298

Prussin, A.J., Schwake, D.O. and Marr, L.C. (2017) Ten questions concerning the aerosolization and transmission of Legionella in the built environment. *Build Environ.* 123: 684–695.

Spartan cube official product page, https://www.spartanbio.com/cube, Last Access: 22 October 2019

Tim Sandle, T. (2015). Settle plate exposure under unidirectional airflow and the effect of weight loss on microbial growth. *European Journal of Parenteral and Pharmaceutical Sciences*. 20(2): 45-50

Yamaguchi, N., Tokunaga, Y., Goto, S., Fujii, Y., Banno, F. and Edagawa, A. (2017). Rapid on-site monitoring of Legionella pneumophila in cooling tower water using a portable microfluidic system**.** *Scientific Reports*. 7: 3092