LamdaGen's L'AuRa Platform for Rapid Point of Care Diagnostic Assays

Legionella pneumophila Serotype I

February 2019

1455 Adams Drive, Suite 1155, Menlo Park, CA 94025 USA
P: 650.571.5816 F: 650.571.5837 www.LamdaGen.com

BACKGROUND

Legionnaires' disease is a severe form of pneumonia, which is often lethal to older people and those with compromised immune systems. It is caused by the bacterium *Legionella* found in both potable and non-potable water systems. Each year, an estimated 56,000 to 113,000 people are infected with the disease in the United States, with the global incidence being far higher and increasing, partially due to rising geriatric populations in Asia and the US. Legionnaires' disease requires intensive care and hospitalization; hence, it is considered a public health concern. Legionnaires' disease was first recognized in 1977 after an outbreak among attendees at the 1976 American Legion convention. In 2016, Health departments in the United States reported about 6,100 cases of Legionnaires' disease[1] with a mortality rate of about 10%.[2] The official numbers may underestimate the true incidence of the disease because Legionnaires' disease is likely underdiagnosed due in part to a lack of convenient testing methods.

The global Legionella testing market is experiencing considerable growth due to factors such as rising incidence of pneumonia and Legionella-related illnesses. In 2016, the global legionella testing market size was valued at USD 180.0 million, and is projected to grow at a compound annual rate of approximately 9.4% during the forecast period 2017-2025.[3] In the US, during 2016-2017, the number of Legionnaires' disease cases increased 13.6% over the previous year. Similarly in Europe, during 2011- 2015, the overall notification rate for Legionnaires' disease rose significantly.[4] The increasing incidence of Legionella-related disorders and rising awareness about preventive management are expected to drive growth of the Legionella detection market.

Legionella is present naturally in fresh water environments, such as lakes, reservoirs and streams where it does not represent a major threat because the background level is low. Moreover the bacteria can remain dormant for extended times under a wide range of environmental parameters. However, when airborne, *Legionella* can become a health concern. People can contract Legionnaires' disease when they breathe in small droplets of water (aerosols) that contain the bacterium. In general, the disease is not spread from person to person although this may be possible under rare circumstances.[5]

Aerosols can be produced anywhere water is splashed or sprayed. Some of the most common sources associated with Legionnaires' disease are cooling towers, air conditioning units, water fountains, whirlpool spas, showers, faucets and car washes. These potential sources of contamination are found in many private and public places such as hotel, schools, hospitals and cruise ships. It is also more commonly an issue in Europe where water systems are older.[4] However, as structures in the US age, the risk of contamination is also increasing.[6] Under the right conditions, *Legionella* bacteria can double in number every 90 minutes, meaning that a very low dosage of bacteria (mostly inoffensive for human health) could increase thousand fold in one or two days. Such a scenario can result in an outbreak that poses a major health risk.

The genus *Legionella* is a pathogenic group, gram negative, non-spore forming bacteria that includes *Legionella pneumophila (L. pneumophila),* a single species found in over 90% of *Legionella* infections. Among this species, the serogroup 1 causes more than 80% of all infections and is directly implicated in all major outbreaks. All major test offerings detect the presence of the *L. pneumophila* serotype I as a proxy of a potential risk of disease exposure.

CURRENT TESTING METHODS & LIMITATIONS

Current testing methods for the presence of *L. pneumophila* in water systems include PCR or similar molecular methods and immuno-chromatographic assays. While the molecular methods are more sensitive, they require trained personnel and the infrastructure of an offsite central laboratory

to perform the test in a cost-efficient manner. The results often are not available for days following sample collection, thus leaving in limbo the confirmation of a potential *Legionella* outbreak.

On the other hand, immuno-chromatographic assays or Rapid Lateral Flow Assays (RLFA), are exquisitely simple to run and yield results in a matter of 25-30 minutes.[7] They detect the presence of cell surface antigens from *Legionella* bacteria in a given sample. However, RLFA suffer from poor sensitivity where their limit of detection is typically about 10^6 *Legionella* copies per milliliter of solution.[6] They often catch the *Legionella* outbreak too late, when the bacterium has already been able to multiply to millions of copies and potentially spread to other parts of the water system.

L'AURA – THE NEXT GENERATION OF RAPID POC TESTING

LamdaGen has developed L'AuRa, a platform that combines the speed and simplicity of current RLFA with the sensitivity of microtiter plate assays (more than 2 logs below the LOD of RLFA). The ability to detect a lower bacteria count at the source may prevent an outbreak from occurring in the first place. Consider the example of a hotel with hundreds of rooms to monitor daily. If we assume that sampling is performed once a day and that bacteria counts double every 90 min, lowering test detection levels by ~2 logs (LOD at 10^4 vs 10^6 cfu/mL) may lead to identification and isolation of a contaminated area as much as a full work-day earlier than currently possible.

LamdaGen's L'AuRa core technology is based on the color change of its nanostructured thin-film sensors induced by a specific target binding on the thin-film's surface. The color of the film is captured using a digital camera, very similar to the ones used in any modern Smart Phone. The associated color change is imperceptible to the naked eye, yet it leaves a measurable imprint on the digital image captured by the camera. This subtle color change is precisely quantified through powerful proprietary AI algorithms partially inspired by modern astronomy techniques using space- and ground-based telescopes for discovering and characterizing exo-planets and exo-moons.[8]

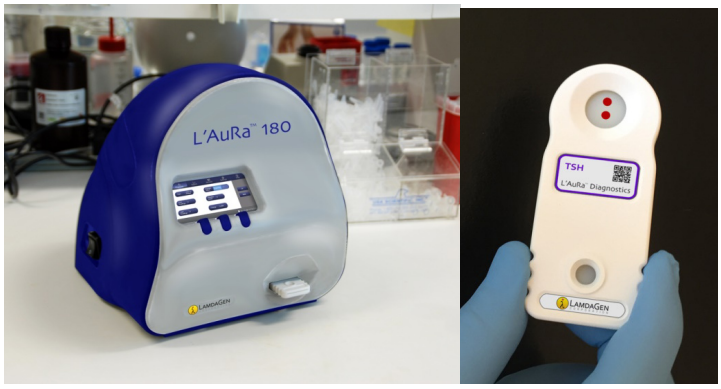

Figure 1: LamdaGen's small benchtop L'AuRa 180 digital reader (left) and cartridge (right) prototypes for rapid and quantitative POC diagnostic testing.

The cartridge flow cell core is made of specially selected polymers with properly balanced hydrophobicities. The L'AuRa 180 reader and cartridge system do not require any moving parts and are amenable to POC and handheld mobile applications.

LamdaGen's technology is unique due to a seemingly innocuous yet remarkable property of the nanostructured films. First recall that all existing POC technologies require signal amplification which is often performed using fluorescent dyes or enzymes linked to a secondary antibody. Excess dye or enzyme conjugates must be washed away in order to read the amplified signal. L'AuRa also uses a proprietary amplification conjugate in its immunoassay which is designed to interact with the plasmonic surface. The chemical moiety conjugate greatly enhances the color change of the surface, however *it does not need to be removed* in order to read the assay. L'AuRa leverages several unique and remarkable properties of the nanostructured thin-films, namely an exceedingly short sensing range (only 20-40 nm) that voids the need for a wash step. Conjugates that are further away from the surface are outside of the sensing range and invisible to the surface and thus do not need to be removed. Only the conjugates that *truly bind* to the surface due to the presence of the antigen actually come into the sensing range and affect the color of the surface.

The combination of the moiety conjugate and the nanostructured thin-film enables deep limits of detection in a single-step assay (no wash). This no-wash step feature considerably simplifies the cartridge design and allows for inexpensive diagnostic system engineering **with no moving parts or complex fluidics**. Since there is no need for reservoir packs, pumps, valves or actuators to drive fluids, the design and engineering of the flow cell are very simple, robust and cost-effective. Additionally since no wash-step is necessary, the user simply adds the sample to the cartridge inlet and waits 5-20 minutes for test results to be computed, displayed and/or uploaded.

L'AURA AND LEGIONELLA PNEUMOPHILA DETECTION AT 10^4 CFU/mL IN 20 MIN

L'AuRa has been used for the detection of *Legionella pneumophilia* serotype I in a 20 min single-step assay. 50 µL of sample containing various amounts of the antigen is premixed with the antibody-moiety conjugate and added to the inlet port of the cartridge. The sample is immediately drawn through the channel to the sensor chamber by capillary action. Immediately after the sample has filled the sensing area, an image of the sensor spot is captured. After 20 min, a second image of the same area is captured again. Both images are input into LamdaGen's proprietary algorithms that precisely evaluate the change in color intensity between both images. The algorithms produce a false-color image that highlights the areas of the sensor where color changes occurred.

An example of the resulting false-color image is shown in Figure 2, where assays at various antigen concentrations are presented. Pixels with a red-orange color represent areas with little color change and blue pixels represent those with a larger degree of color change. It is strikingly apparent that only areas of the sensing spots (the half circle in the lower part of the image) exhibit a color change, while the top part of the image (outside of the sensing spots) does not. In fact, all samples containing the antigen show a color change in the sensing spot. Thus, detection of 10^4 pathogen per milliliter in 20 minutes is confirmed visually. This level of detection represents 2-logs of increased sensitivity improvement over commercial rapid lateral flow strips (LOD ~ 10^6 pathogen/mL in 25-30 min assay).[7]

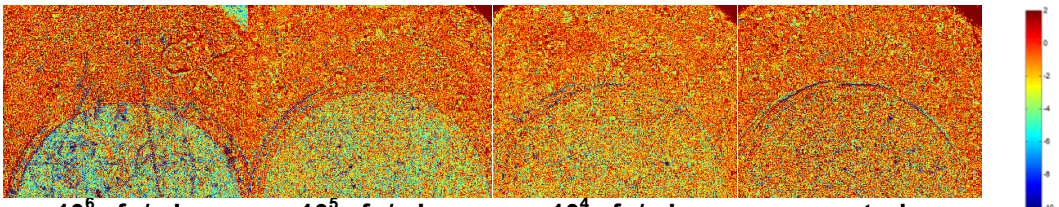

Figure 2: Examples of assay results for samples with various amount of *Legionella pneumophilia* serotype I antigen. The control (far right) has none of the antigen, while the other panels have various amount of the antigen (labeled above the image). The false-color images map the locations where the color changes from the moment the sample contacts the sensing spot (time t_{ini}=0) and a certain amount of time later (here t_{assay} = 20 min). The color map is such that the bluer the pixel, the larger the color change. Notice how only the sensing spots undergo a color change and furthermore, the larger the *L. pneumophilia* antigen concentration, the larger the color change.

The amount of color change can be quantified in a very precise manner. For instance, the algorithms score the change in color within the sensing area and each assay is ascribed a score or readout. Table 1 reports the readouts of assays for various dosage of the antigen, along with the threshold levels for the margin of confidence (1σ is 68%, 2σ is 95%, 3σ is 99.7%). Figure 3 plots the graphical dose response of Table 1. Both confirm the detection at the 10^4 pathogen per mL above the 3σ threshold, i.e. with a level of confidence greater than 99%.

Table 1 *(below, left)*: Readouts computed by the algorithm after 20 min of incubation for various titers of *L. pneumophilia* serotype I. For each titer, the average values of all runs (N=3) along with the standard deviations are reported. Also, we report the threshold levels for positive identification. The thresholds are computed as $m_o + X\,\sigma$ where m_o is the average of all controls, σ is its standard deviation, and X = 1, 2 or 3. We interpret the 3σ threshold level as the readout level above which the probability that a readout represents a true positive is greater than 99.7%.

antigen concentration [cfu/mL]	Readout @ 20 min	
	average	*std*
0	0.091	0.027
10^4	0.192	0.056
10^5	0.51	0.051
10^6	1.02	0.082
Thresholds for detection		
1σ	0.118	
2σ	0.145	
3σ	0.172	

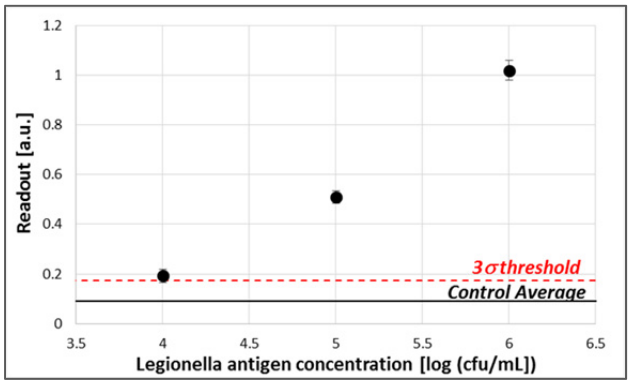

Figure 3: Graphical representation of Table I; the concentration is plotted on a logarithmic sale

LEGIONELLA PNEUMOPHILA DETECTION IN A BLIND STUDY

To further validate the L'AuRa platform for the *L. pneumophilia* serotype I assay, a blind study using a limited number of samples was performed. N=10 samples were prepared at various titers and tested by an independent operator. The operator did not know the nature of the sample and was tasked with providing 1) +/- call and 2) an estimate of the amount of Legionella in the sample.

The operator set the threshold for +/- call at 10^4 pathogen per milliliter of solution, consistent with a greater than 99% confidence in each call (Table 1). Based on the readout value, the operator estimated the amount of *Legionella* based on the dose-response curve of Figure 3. Table 2 summarizes the blind study results. One sample was miscalled (sample 6). The comparison between the titers called by the operator and the true values also show a remarkable agreement.

Blind Sample	Operator call	TRUE	Operator titer *	TRUE *
1	+	+	4	4
2	-	-	0	0
3	+	+	4	4
4	+	+	6	6
5	+	+	4	4
6	+	-	4	0
7	-	-	0	0
8	-	-	0	0
9	+	+	4	4
10	+	+	5	5

Table 2 Blind study results in a single-step 20 min assay using L'AuRa. The operator called 9 out of the 10 blind samples correctly. Also, notice that the only false-positive call concerns sample 6, which was a titer 100-fold lower than what current RLFA can detect.

*The number refers to the logarithm of the concentration; hence 4 indicate a titer of 10^4 cfu/mL.

LOD for L'AuRa: < 10^4 cfu/mL
LOD for RLFA: 10^6 cfu/mL

CONCLUSION

We have presented the results of the L'AuRa *Legionella pneumophilia* serotype I rapid assay, detecting the strain of the bacterium responsible for over 80% of Legionella infections. The L'AuRa platform enables a no-wash step, quantitative assay and does not require any user intervention besides the addition of the sample into the cartridge inlet.

The 20 min assay has a limit of detection below 10^4 bacteria per milliliter of solution and only requires 50 µL of sample. L'AuRa also provides the option to quantify the amount of antigen in the sample, a benefit that could help estimate the moment an outbreak occurred, i.e. a larger readout indicates more division cycles of the bacteria; knowing the division rate, it may be possible to pinpoint the time of the original contamination.

The performance parameters of the current L'AuRa assay (20 min assay, 50 µL sample, LOD 10^4 cfu/mL) greatly outperforms commercial rapid lateral flow assays. In particular, the LOD of the L'AuRa assay is more than 2 logs lower (more than 100x) than that of current RLFAs. Given the division rate of this bacterium is about 90 min, such improvement suggests that enrichment cycles may be reduced by more than 12 hours prior to running the assay. The current L'AuRa LOD makes it possible to detect a *Legionella* outbreak a mere 18-20 hrs post contamination when a ***single*** bacterium enters a system, and in less than a work-day shift if the contamination is initiated with a few hundred bacteria.

REFERENCES

1. Centers for Disease Control and Prevention (**2017**). National Notifiable Diseases Surveillance System, 2016 Annual Tables of Infectious Disease Data. Atlanta, GA. CDC Division of Health Informatics and Surveillance. Available at: https://wonder.cdc.gov/nndss/static/2016/annual/2016-table1.html

2. Dooling KL, Toews KA, Hicks LA, et al., Active Bacterial Core surveillance for legionellosis–United States, 2011–2013. *Morbidity and Mortality Weekly Report*. **2015**;64(42):1190–3.

3. Transparency Market Research (March 2018), Legionella Testing Market - Global Industry Analysis, Size, Share, Growth, Trends and Forecast 2017-2025 — https://globenewswire.com/news-release/2018/03/14/1422192/0/en/Legionella-Testing-Market-is-estimated-to-bring-US-398-7-million-revenue-by-2025-TMR.html

4. Beauté J, on behalf of the European Legionnaires' Disease Surveillance Network. Legionnaires' disease in Europe, 2011 to 2015. Euro Surveill. **2017**;22(27). DOI: http://dx.doi.org/10.2807/1560-7917.ES.2017.22.27.30566

5. Correia AM, GonCalves J, Gomes, JP, et al., Probable person-to-person transmission of Legionnaires' disease. *N Engl J Med*. **2016**;374:497–8.

6. Bruce Y. Lee, Legionnaires' Disease: Why Is This Potentially Deadly Disease Seemingly On The Rise?, in Forbes, 3 Sept. 2018 — https://www.forbes.com/sites/brucelee/2018/09/03/legionnaires-disease-why-is-this-potentially-deadly-disease-seemingly-on-the-rise/#46cd114968d0

7. See https://hydrosense-legionella.com/legionella-test-kits/ and the instruction notice herein (accessed January 2019).

8. Lee Billings, (3 October **2018**), Astronomers tiptoes closer to confirming first exomoon, Scientific American — https://www.scientificamerican.com/magazine/sa/2018/10-01/