CONCURRENT SESSION 3 – BIOLOGICAL AGENT SAMPLING & ANALYSIS METHODS

Questions and Answers

- **Anonymous:** Question for Ernest: How are viruses aerosolized and is the fluid used for aerosolization that surrounds the viruses a barrier for inactivation?
    - **Ernest Blatchley, XCMR Inc.:** Great question. In the experiments that we conduct in the laboratory, we aerosolize using a device called a nebulizer – you can think of this as a spray-paint can – so you take a liquid, and you project it as really small droplets. The nebulizer we are using is specifically designed to release droplets that are about the same size as those released by a human when they are talking/coughing/sneezing – at least the droplets that we are really concerned about – the aerosols that are going to stay suspended in air for a long period of time.
- **U.S. EPA:** Question for Ernest: Do material compatibility issues need to be considered with any of your potential use cases for continuous use on surfaces?
    - **Ernest Blatchley, XCMR Inc.:** Yes; material compatibility is definitely an issue. There are several materials that are largely inert to exposure to UV, but there are some, including some plastics that will degrade because of exposure to UV. So yes, we would need to be sensitive to that and take it into account in the design.
- **Anonymous:** Question for Paul: What is the bag material made of? What polymer?
    - **Paul Lemieux, U.S. EPA:** I am not exactly sure what the polymer is; I think it is a combination of multiple materials that are layered together. I am not exactly sure what the materials are. I do not know if anyone else on the call could elaborate or I can follow up with someone.
- **U.S. EPA:** Question for Paul: The current material has pore sizes between 20 and 60 microns. Do you think they will be able to reach your goal of 1 micron pore size?
    - **Paul Lemieux, U.S. EPA:** I am pretty sure they will be able to reach the goal of the target pore size. The question is whether or not the time it takes for fumigating the roll-off (or whatever container we have the bags of waste in) – how long we will have to carry on the fumigation in order to achieve the required Ct numbers inside all of the bags so that the spores can be properly inactivated. That will be one of the key elements of testing once they send us the material.
- **Anonymous:** Question for Scott: Wouldn't a lower delta Ct imply more of the DNA present initially?
    - **Scott Nelson, Battelle Memorial Institute:** The way that the Ct values are calculated is you take the pre-enrichment Ct value (qPCR value) and the post-enrichment Ct value. And for a sample that has a lot of target present after enrichment, the Ct value is typically somewhere around 20Ct – it crosses the threshold at 20 – whereas the T0 aliquot or the pre-enrichment aliquot typically is undetected in the thermo-cycler; you do not get a Ct value generated. In those cases, we assign a Ct value of 45, which is the total number of cycles run in the PCR. Then we take the pre-enrichment value, and we subtract the Tfinal. So, in this example: 45 (for an undetected pre-enrichment Ct value) minus Ct value of 20 (for a sample that had a large degree of growth during enrichment) and that value is 25 (delta-Ct of 25). If it had a low Ct value

for Tfinal (say, something at ~30) then 45-30 you get 15. So higher delta-Ct value means more enriched spores or target.

- **Anonymous:** Question for Scott: How would spore loss occur if samples were not exposed to sun, rain, or wind?
    - **Scott Nelson, Battelle Memorial Institute:** So, our surfaces were stored in an outdoor shed, which provided some protection. However, there was a blower motor and some windows, so that particulates, and there was a continuous air flow. There could have been spore-loss due to that. Other consideration of course is maybe our recoveries or samplers were not picking up as many spores after background grime and dirt material accumulates over the top – that also impacts our level of spores that we're seeing in our analytical methods.
- **Anonymous:** Question for Ernest: It seems that the far UVC lamp you have been using inactivates the viruses almost instantly?
    - **Ernest Blatchley, XCMR Inc.:** In the application of UV radiation, the master variable is not time, it is dose. It is true that the coronaviruses, as an example, are very easily inactivated by UV. Practically, what that translates to is that the dose that is required to inactivate those viruses is very small. However, I want to emphasize that what is important, in terms of the application of UV is how much radiation – how much UV – are you delivering to the viruses, will depend on two things: (1) time and (2) what we call fluence rate (which is basically how strong the radiation is in the area that's being irradiated). So, it is not really a question of time, it is really a question of dose and how strong the source of UV is that is irradiating the area. One other comment to add – I mentioned that coronaviruses, including the one that causes COVID-19, are very easily inactivated by UV – it turns out that many of the airborne pathogens are inactivated very readily by UV, so that suggests that devices that are designed to address COVID-19 prevention (in other words, inactivation of SARS-CoV-2) should also be effective for inactivation of a number of other airborne pathogens.
- **Anonymous:** Question for Ernest: If far UVC radiation is safe for humans, how is it possible that the same radiation is so damaging to microbial RNA/DNA?
    - **Ernest Blatchley, XCMR Inc.:** Damage to viruses and bacteria vs. damage to human tissues like the skin or the eye: it is really – at least in my mind – a question of scale. Thickness of human skin and how the far UVC penetrates that layer of tissues, it turns out that far UVC will only penetrate through part of what is called the 'stratum corneum' – that is the outside layer of skin cells on the top of your skin, more or less comprised of dead cells about to be sloughed off. Far UVC does not penetrate through that outer layer and does not penetrate to the layers where skin cells are being developed – the germinative layers. So, because that radiation is absorbed within the proteins of that outer layer of skin, it does not get to the parts of the skin that would cause damage that would be permanent. Then if you compare that, so for perspective the stratum corneum is somewhere between 10-30 microns thick; if you examine an individual bacterial cell its typical size is about 1 micron, and a virus would be a small fraction of the size of a bacterium. What is relevant about that is that the radiation is able to penetrate individual bacterial cells or viruses, but it cannot penetrate through the thickness of the skin, and a similar argument holds for the eye; it just cannot get to the critical parts of either of those tissues to do damage.
- **Lawrence Livermore National Lab:** –Question for Ernest: Interesting technology. Do you recommend eye protection with this approach? I noticed the example photo showed use of eye protection.
    - **Ernest Blatchley, XCMR Inc.:** Yes. For me and anyone else I would recommend eye protection – you only get two eyes! There is certainly evidence to suggest that this radiation will

not cause damage to your eyes, but eye protection is minimally invasive and always a good idea.

- **Anonymous:** Question for Ernest: Could a far UV light device be used to irradiate the nose/mouth/throat to bring down the viral load of a person who has been recently exposed to SARS-CoV-2? If done early enough, it is conceivable that it would lower chance of infection.
    - **Ernest Blatchley, XCMR Inc.:** There is a number of things about that hypothesis that I am unsure about or have trouble with. First, we do not always know when we have been exposed. But even if you do, the tissues that you are talking about (nose/mouth/throat) are fundamentally different tissues than the skin or the eye. I am not aware of any literature that would describe the potential for damage to those other tissue types. I do not have information to inform a decision about whether you would or would not want to use that. My preference would be no since we do not know that information (at least I do not). Another problem is the geometry of those parts of the body is pretty unusual, so being able to control dose (again, the 'master variable'), seems like it would be a little bit difficult in that sort of geometry. Lastly, it is an interesting hypothesis, but I am not aware of any data that would suggest that it is likely to have a positive effect. It really is a fundamentally different question than those we are trying to address with the devices we are developing.
- **Anonymous:** Question for Ernest: What residence time is required to achieve a 4-log kill of a virus?
    - **Ernest Blatchley, XCMR Inc.:** Again, the master variable is dose, not time. The time required to get to 4-log kill depends on how strong the UV source is. For perspective, the UV dose that would be required at 222 to get to about maybe 4-log units of inactivation would be somewhere in the vicinity of maybe 3, or maybe at the outside 4 millijoules per square centimeter. And again, the time required to do that depends on how strong the source(s) are that are delivering far-UVC to the region being disinfected.
- **Anonymous:** Question for Paul: How did your test system generate ClO_2?
    - **Paul Lemieux, U.S. EPA:** One of the slides had the picture of the chlorine dioxide generator. I think it is a Chlordysis system if I am not mistaken. It uses a couple different reagents and produces the required amount of chlorine dioxide. I have not actually been in the laboratory to see it since they set it up because of COVID-19, so I am not exactly sure what the various reagents are but it is a system we have been using for a number of years, though we had to repair it for this project. In practicality, when we do the field test, we are going to have to contract with a commercial firm to bring a chlorine dioxide generator out to the field that will be capable of producing sufficient quantities for a long enough period of time to maintain the fumigation conditions.