COVID Information Commons (CIC) Research Lightning Talk

Transcript of a Presentation by John Yin (University of Wisconsin-Madison), December 9, 2020

Title: RAPID: Ecological Dynamics of Human Coronavirus, EAGER: Rapid and Sensitive Drug Testing for COVID-19

John Yin CIC Database Profile

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YouTube Recording with Slides

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Transcript

John Yin:

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So, I'm John Yin. I'm a professor of Chemical and Biological Engineering at the Wisconsin- at the University of Wisconsin-Madison. I'm also with the Wisconsin Institute for Discovery and I have the honor of sharing some of our work. This is actually ongoing work for the last 15 years that we've pivoted to bring over to coronavirus. It's about turning the dial on coronavirus infections. We have two awards. One of these involves turning up the dial for infections and one involves turning down the dial. I'll try to explain what that means.

Slide 2

I didn't do any of the work. The people who did the work are all here, past and present, many of them now in industry or academia. I think two of my co-workers, Nan Jiang and Huicheng Shi, are on the call with us today. So, these are the people who do the actual work.

Slide 3

To help you understand where I'm coming from, I have to tell you how we quantify infectious virus. So, there's a standard way to quantify infectious virus. So, we have a virus stock solution here that we'd like to figure out how much virus is actually in there? Usually there are millions or tens of millions of particles we would like to figure out how many particles and count them. And the way we do that is we do a series of dilutions, known dilutions, it's kind of watering down the drink until we get a few tubes

that just have countable numbers of particles. Those tubes- known volumes from those high dilution tubes are put on mono layers of cells shown here in the red and overlayered with agar gel, and they are allowed to reproduce. The viruses spread locally and kill the cells. Then we stain the cells blue and wherever this virus has killed the cells there's a little hole there. The hole is called a plaque, a region of dead cells caused by a single virus which we can now see with the naked eye. By counting those holes, or plaques, we can deduce knowing what volumes we had and how much we watered down the original stock, we can figure out how many particles we had in the original stock. So, this is a key tool that we use to quantify infection and that we will use to characterize how we turn down or turn up viruses.

Slide 4

So, the first thing we did here was to try to explore ways that we could get more signal out of this kind of assay and what we did was instead of overlaying with agar so here I show on the no flow cases these are sort of standard plates that you might see petri dishes where they have plaques and you would count those plaques. We carried out the infection in the presence of liquid rather than agar overlay and we found that if you don't touch the plates, spontaneous flows arise- outward radial flows and create comet like morphologies of these plaques. So, there's flow that's automatically happening in there that's helping to spread the virus. And for plates that have the same level of virus, we see a lot brighter signal, so we have a much higher signal to noise. We've done some theoretical models- computational models of this process and in essence we said: this might be a useful tool to characterize drugs. So, what happens if you add drugs against the virus to this kind of signal?

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Here in the top left, here, we have a sample that has no drug in it and then we have five other samples that have different degrees increasing amounts of drug and what you find is the fireworks dim as we go to higher level of drugs. That means we have less and less virus spreading and less infection. We can quantify that here, and on the plot you see a comet enhanced- flow enhanced comet assay versus the plaque reduction assay, which is currently the gold standard. So, our assay is about 20-fold more sensitive and faster to run than the existing plaque assay for drugs. So, this is something that we patented five years ago and with the NIH [National Institute of Health] funding- sorry NSF [National Science Foundation] funding. We are now going on to adapt this assay to test drugs against coronavirus. That was turning up the infection and facilitating the spread. We're also interested in turning down the infection.

Slide 6

How do you dial down virus infections? It may be happening in nature. Here are three scenarios. The first one is the virus infects the cell and it makes a bunch of virus particles. Typically for coronavirus, it makes about 100 coronavirus particles per cell. Among those virus particles, there may be some defective ones. So, the defective ones- this orange as I've shown in the orange here, if it gets into a cell it does not make anything. It's not able to grow. However, the defective particle retains some aspects of

the virus. The ability to use the virus replication machinery. So, if both an intact virus shown here in blue and the defective virus shown in orange get into the same cell as a co-infection then you can have the defective virus reproducing, stealing resources from the growth of the normal virus. So, these defective particles are called defective interfering particles because they interfere with the normal growth of the virus. They've long been known since the 1950s, and in the 1970s I've taken this abstract from this review article- very prominent review article in 1970. So, 50 years ago, people were speculating that these might play some role in viral diseases. So, building on that, we thought it would be interesting to try to quantify interference. How do you quantify interference?

Slide 7

Well, we set up something like the plaque assay and the details here are not so important other than we had to make some dilutions and instead of providing viruses- instead of providing live cell to viruses the way we do for the plaque assay, we have to provide infected cells to defective particles. That's what the defective particles prey on.

Slide 8

So, if I go to the next slide we can see the data such that as we go to higher levels of these defective interfering particles going from left to right on the x-axis here the virus production drops, and then comes back up. If we look at the production of the dips of the defective particles- how they depend on their own input levels, we have this other kind of behavior that's increasing up to a point and then dropping off very sharply. So, the key point here is that dips exhibit a complex behavior with respect to their ability to inhibit virus growth, and also with respect to their ability to influence their own replication. So, to better understand this, we have studied a different kind of virus, a rabies-like virus and we've engineered two kinds of virus. One that is carrying a red fluorescent protein, so when it infects cells, it turns the cells red, and one that's a defective particle that carries a green fluorescent protein.

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So, the scenario is here. There are two different viruses. One is the virus red and one is the defective virus green. And what we do is infect a single cell with both of those and then watch how they spread over several generations. So, in this dish, you can see at three hours post infection, you see no fluorescent protein and if you watch the top right-hand corner you'll see the time ticking off as we take images at various times. This is 300 microns or about a third of a millimeter. Okay, so watch the timer and watch the patterns.

This is our pandemic in a petri dish. So, millions of cells are being infected from that single one virus particles and defective particles released from that cell are spreading over this time period of less than a day or so. So, we have used this to study in depth what's happening at the single cell level and these sorts of patterns then you'll see are quite complex. It's really a predator-prey behavior in the sense that the defective virus is preying on the infected cells.

Slide 10

If we look at the extent of normal virus spread in the absence of any defective particle, we can see the red expanding. In the presence of defective particle alone, there's no infection, but in the presence of both we get a co-infection spread that is inhibited relative to the normal virus. So, we wonder could defective coronavirus be used to treat COVID-19 in a way to inhibit or slow down the spread?

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So, today I've told you about two cases where we dial up infection spread and this is good for antiviral testing because it gives us a more sensitive assay, and I've also talked about dialing down infection. There are natural particles that arise from these infections that can inhibit the growth and spread of viruses. We hope to investigate this for reducing the severity of COVID-1, COVID-19 I'm sorry that should be 19. If you'd like to learn more, I'd be happy to respond to chat questions or email. Thanks very much.