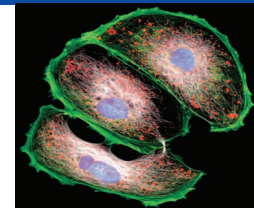


Studying the Viral/Bacterial Connection, One Cell at a Time



Interview with Colette Cywes Bentley, PhD, Assistant Professor of Medicine, Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School

About Dr. Cywes Bentley

Dr. Colette Cywes Bentley serves on the faculty of the Harvard Medical School in Boston, Massachusetts. She is a principal investigator at The Channing Laboratory, a multidisciplinary research division of Brigham and Women's Hospital and Harvard Medical School. The Laboratory investigates bacteriology, chronic disease epidemiology and virology. Dr. Cywes Bentley has conducted research at The Channing Laboratory since 1997. She began using the AmpliGrid platform and AmpliSpeed cycler from Advalytix AG in May 2007.

In her research at The Channing Laboratory, in the heart of Boston's medical research community, Dr. Colette Cywes Bentley faces numerous challenges every day. Isolating and investigating cells of interest at the single cell level is no longer one of them.

As part of the bacteriology group at the Channing, where she is the principal investigator on research exploring the clinically identified relationship between varicella zoster virus and Group A *Streptococcus* in necrotizing fasciitis, Dr. Cywes Bentley is driven to determine what the virus does to the host cells to facilitate aggressive bacterial super-infection. In her current research, she is studying how the childhood Chickenpox virus and the bacteria commonly known to cause strep throat synergize to lead to a rare but sometimes fatal skin infection.

"I am interested in understanding what viruses do to predispose us to getting bacterial superinfections," said Dr. Cywes Bentley, noting there are many clinical associations between viruses and bacteria, including human papillomaviruses and Chlamydia and influenza and *Streptococcus pneumoniae*. "How do viruses change our epithelium, in this case the skin, so that the bacterial infection is so much more severe? What is the virus doing to the cells to promote bacterial virulence?"

The answer, in part, lies in her ability to look at individual cells. Prior to employing the AmpliGrid system from Advalytix for single-cell analyses, Dr. Cywes Bentley did what many researchers do – she infected her cell samples, extracted RNA from each well, amplified the genes of interest, examined the results and drew inferences based on the entire sample population. However, the samples are not homogeneous – not every cell in the sample is infected. In some wells, more cells were infected with virus than others skewing the results.

"When I infect a well of one million cells with varicella, I'm aiming for 80 to 90 percent infection, but each day is different," she explains. "Perhaps only 70 percent of my cells get infected. If I harvest those cells on the premise that 90 percent are positive...you get a misrepresentation of what the virus is doing to the cell population as 30% of the transcripts will be derived from uninfected cells. The AmpliGrid gives us the opportunity to dissect out how each cell is responding to the virus, bacteria, or both the virus and the bacteria if dual infected."

Single-cell analysis enables Cywes Bentley and other researchers to isolate and investigate exactly the cells of interest, one cell at a time, reducing misrepresentations in the results. Unlike entire cell population analysis, the AmpliGrid platform allows the capture of single cells on a chemically structured microscope slide by sorting directly onto the AmpliGrid slide using flow cytometry. Cywes Bentley explains that, rather than study her sample as one population, using the AmpliGrid she is able to sort her sample into four possible populations: uninfected cells, varicella-infected cells, Group A Strep-infected cells, and dual varicella- and Group A Strep-infected cells.

"We were taking the entire cell population in the dual virus and bacteria infected wells, which in reality consists of all four cell populations, and studying them together as one population to see how the cells were responding to dual infection," she said. "Now, if every cell were infected with both virus and bacteria, you'd have a nice homogeneous population, but that's not the case. In a mixed population, the signal can vary depending on the various cell population proportions."

She continues: "I was artificially using a well that I had infected with both varicella and Group A Strep as being all dual-infected, when we know by looking with microscopy that it's a mixed population. We're concerned that even though only a small percentage of the cells in the mixed population aren't infected or are only singly infected, they may skew or mask the results from the cells of interest. Single cell analysis cleans this up for you."

Using the AmpliGrid system, Cywes Bentley is confident in the accuracy – and reproducibility – of her results.

"This leads to consistency of results because the population is homogeneous versus heterogeneous. In these experiments it was a problem of heterogeneity."

Continued on reverse.

“It’s incredibly frustrating to use exactly the same viral inoculum, time course, and bacterial inoculum and get varying results each time,” said Dr. Cywes Bentley. “By analyzing single cells from each separate population, there can be reproducibility.”

Not only that, Cywes Bentley said inconsistency of results after following the same experimental methodology is “incredibly frustrating.”

“It’s incredibly frustrating to use exactly the same viral inoculum, time course, and bacterial inoculum and get varying results each time. It’s not possible to consistently get the same number of bacteria associated with the same number of virus-infected cells in the well each time, even though you’re harvesting the same number of cells. By analyzing single cells from each separate population, there can be reproducibility.”

Dr. Cywes Bentley’s current research uses single cell analyses to isolate and separate cells of interest from neighboring cells that may obscure the signals from these cells. She is using single cell analysis to compare and contrast cells from each population in an infected sample. Eventually, she will apply single cell analysis to determine differences within like-cells – studying the differences within all varicella-infected cells, or cells dually infected with varicella and Group A Strep.

The distinction in separating populations is important to Cywes Bentley. She points to research investigating pStat1 expression in varicella lesions (Ku, C.C. et al, J Exp Med. 2004 Oct 4; 200(7):917-25). Results indicate the varicella-infected lesion contained high levels of pStat1, when in fact, pStat1 was shown to be decreased in varicella-infected cells and that the increased signal was originating from the neighboring non-infected cells.

“That’s what’s been technically very difficult for me – that the neighboring uninfected cells overwhelmed the infected cell signal. Being able to tease these cells apart and look at the single cell level has just been fascinating.”

That separation is critical to Cywes Bentley’s understanding of why the bacteria target certain cells and what role the virus plays in the dual-infected cells.

With AmpliGrid, “I’m able to get a better handle on the questions I’m asking. It allows me to focus on the population of interest, without background noise – non-specific background noise overwhelming the signal.”

Prior to being introduced to AmpliGrid and single cell analysis, Cywes Bentley says she didn’t approach her research by thinking about separating her mixed or multiple cell populations.

“But when you have a tool you didn’t have before, suddenly you start thinking in different ways.”

And making important discoveries.