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# Delving into the murine immune system with 1 µL of sample:

# A conversation on the expanding horizons in mouse model research

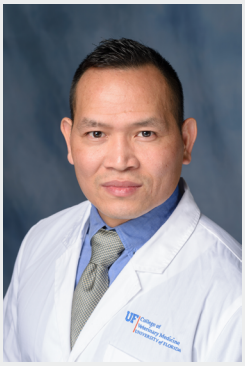
In this engaging interview with field expert Cuong Nguyen, Associate Professor at Department of Infectious Diseases and Immunology at the University of Florida, we explore the significance of simultaneously measuring key immune mediators with high sensitivity, enabling good detectability of low abundance biomarkers.

This approach has the potential to revolutionize our understanding of autoimmune diseases such as Sjögren's syndrome, where limited biological samples are the norm. By employing state-of-the-art technology capable of multiplex detection, researchers can now extract comprehensive data from volumes as low as a 1 µL.

The small sample volume allows using the same sample aliquot for various tests, so that researchers can draw more confident associations between immune responses and disease stages.

In addition,  there are particular advantages in longitudinal studies, potentially reducing the number of animals needed for research and allowing for real-time monitoring of disease dynamics and treatment responses.

This conversation illustrates how leveraging small sample volumes with sensitive multiplex cytokine measurements can pave the way for targeted therapies in autoimmune diseases and beyond.



"Having a comprehensive panel that can handle small volumes with high sensitivity is a game-changer for studying disease. We no longer need to focus on one or two cytokines at a time. We can look at a whole bunch of them and then extrapolate the entire immunological and clinical process."

Dr. Cuong Nguyen,
Associate Professor at Department of Infectious Diseases and Immunology at the University of Florida

## Could you provide a brief overview of your current research focus and its importance in understanding Sjögren syndrome?

Basically, Sjögren's syndrome is an autoimmune disease in which the immune system attacks the lacrimal and salivary glands. Patients with this disease cannot produce saliva or tears, and this dryness in their system is highly detrimental to their health. My research focuses on several aspects: We examine how T cells and their receptors function, particularly in terms of recognizing different auto-antigens, and what happens once activation occurs. This is why measuring the cytokine and chemokine levels of different cell types is important to us. Once we determine the responsible antigen and T cell receptors, we design small molecules to block these interactions. Essentially, if we can block the interactions between an antigen and a T cell, we hope to prevent the pathogenic cascade and halt the disease process. Future work will concentrate on designing specific molecules to block the HLA interaction with the auto-antigen, aiming for a future where we can personalize treatment for different patient populations.

## How do animal models help elucidate the pathogenesis and potential treatment avenues for Sjögren syndrome?

Our lab is one of the first labs that developed different models to look at the Sjögren's syndrome disease process. We use a spontaneous mouse model that very much mimics what happens in patients. With the model that we developed in the lab, we can identify the precise mechanism from the initiation to the full-blown clinical disease. The mice go through different stages. At an early stage, we learn about the immunological processes leading to the disease, how the immune system is evolving, and lastly, what impact it has on the clinical stage. Having a mouse model allows us to learn about different disease stages which we cannot see in humans. In patients, we have access to the very end stage of the disease, and you never know what actually initiated the whole process. With the mouse model, we can understand the disease process really well. Because of that, we can design targets that can prevent the early stage, the immunological stage or the clinical stage.

## Which murine sample type do you predominantly use in your studies?

We use saliva and tears as the main fluids in our study, analyzing gland lysates to fully understand their contents. The samples we collect are very small in volume, making it essential to have an assay that accommodates this.

## Are there specific cytokines or immune mediators that are especially relevant for Sjögren syndrome research?

It's a good question. The beauty of what we do is that we can correlate the human and the mouse data. The mouse data shows that different cytokines and chemokines play different roles at different stages. We learned that at an early stage we observe a lot of IFN-α, β, and γ. In the middle stage, at the immunological response, we see cytokines such as IL-4 and IL-10. IL-4 is required for B cell activation and IL-10 is involved in regulatory T cells. The balance of these and other cytokines will determine the activity of immune cells leading to the clinical stage. When we get to the clinical stage, we see the involvement of Th17 cells, so we measure IL-17A, IL-17F, IL-23, and IL-27. These cytokines are involved with the destruction of the glands. We can pinpoint the exact cytokine and chemokine needed for immune cells to enter the gland and affect its function.

## Are the relevant immune mediators easily detectable in your chosen sample type?

This is something with which we have struggled for a long time. While we can measure high levels of IFN-γ and TGF-β in mice, we see low detectability of Th17 cytokines such as IL-17A, IL-17F, IL-23, and IL-27. An assay with high sensitivity allows us to answer many questions and opens up new areas of research. When I first started doing this work, the only way I could detect IL-17 was using immunohistochemistry or RT-PCR. Now, we have assays that can detect above a certain threshold.

## Can you describe some challenges you've encountered when trying to measure cytokines in multiplex?

We cannot collect large volumes of saliva, tears, and blood from mice. For example, we probably get less than 50 microliters of sera. We get around 20-30 microliters of saliva, specifically at the clinical stage, and even less for tears. In addition, we usually need to freeze the samples before running assays. After freeze and thaw, I would say that more than 50% of the samples typically fail when performing most of the currently available commercial assays.

## What is your experience with Olink Target 48 Mouse Panel? What are key advantages of this panel and can it help overcome previous limitations in your research?

We sent 40 samples to Olink, and 38 out of 40 samples passed the assay QC criteria. That is a 95% success rate, which is remarkable. We have never had such results before. The data we obtained show great sensitivity; we could measure down to 0.5 pg/mL for some cytokines. Because of that, we can use this data to extrapolate pathways and biological processes. What is also cool is that we obtained all this data using a very small volume of our samples. As a result, we ran additional experiments on the same sample, such as auto-antibody profiling. That allows us to correlate the cytokine data with auto-antibody production, which is critical for Sjögren's Syndrome. We would not have been able to run these kinds of studies before because we wouldn't have had enough sample volume to conduct different kinds of experiments from the same sample.

## Will the minimal sample volume be advantageous for the design of longitudinal studies? Do you see any value in running true longitudinal studies in your research?

Yeah, yeah. When you extract blood from a mouse, you don't want to bleed them too much. You can probably get up to 20 to 50 microliters. If you want to follow that same mouse over time, it is difficult. We recently started working with a drug company, and they want to treat the animals and follow them over time to see how effective the drug is at inhibiting different aspects of the disease at different stages. They also want to determine the bioavailability of this drug. So, having this comprehensive panel that can handle small volumes with high sensitivity is a game-changer for studying disease. We no longer need to focus on one or two cytokines at a time. We can look at a whole bunch of them and then extrapolate the entire immunological and clinical process.

## And by doing that, is there any room to reduce the number of animals that you need for these longitudinal studies?

Yes, that is a great question. Of course, if I get a 98% success rate for every sample I submit, I don't have to use 10-20 mice per group, I can use five or six to achieve the statistical and biological significance. This is part of the reduction in the three Rs for the ethical framework of animal experimentation. You don't want to use too many mice; you want to use the minimal number of mice that will give you the best data. So, I had actually never thought about that, but yeah, you're right.

## Are there any exciting developments or upcoming projects in your lab related to Sjögren syndrome that you'd like to share with our audience? Will the Olink Target 48 Mouse Panel play a role in these endeavors?

We have two projects that we are working on right now. We created a humanized mouse model that has the HLA of a human in the mouse. This specific HLA has been associated with Sjögren's syndrome, providing the full spectrum of the disease. These mice have the same auto-antibody profile, lose saliva and tear flow rate, and have immune cells in the gland. It is a great model for testing drugs that target the HLA to see if we can stop the whole cascade event. We plan to use the Olink Target 48 Mouse Cytokine panel to assess if treatment normalizes the cytokine and chemokine levels and if it changes the pathogenic T cells we found in Sjögren's.

Another thing that we are looking at is how humans and mice infected with SARS-CoV-2 can develop Sjögren's syndrome. Similarly, our plan is to determine how viral infection can induce cytokine and chemokine changes that can modulate the immunological impact on the salivary and lacrimal glands and, eventually, the development of Sjogren's syndrome in the mice.

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