Design for Biosecurity

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Lecture 27

Sandwich (Non-Competetive) Assays

Welcome back to the sixth week of this course. We are now into the second lecture of this week. In our previous session, we delved into one of the most prominent assays, exploring how lateral flow immunoassays (LFIAs) function. When discussing immunoassays in general, the underlying logic is quite straightforward. For instance, consider a scenario where you have a toxin. To detect this toxin, antibodies or specific markers are raised against it. These markers are then immobilized, which is a crucial step, particularly for the immobilized plate, where the toxin is rolled over.

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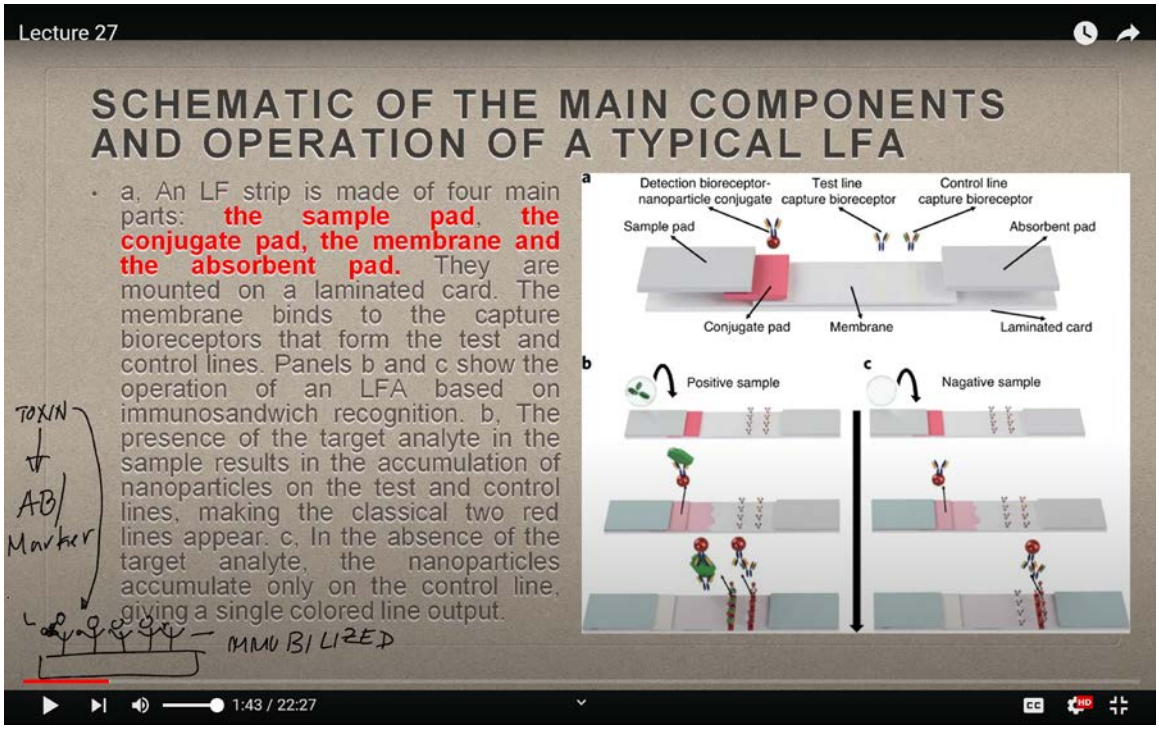

When the toxin binds to the substrate, it can be detected in multiple ways. For example,

binding may induce a conformational change in the toxin, which can then be measured. Alternatively, the binding might activate a fluorescent marker. However, there are other detection methods, such as competitive binding, which is why we discussed it in the last lecture.

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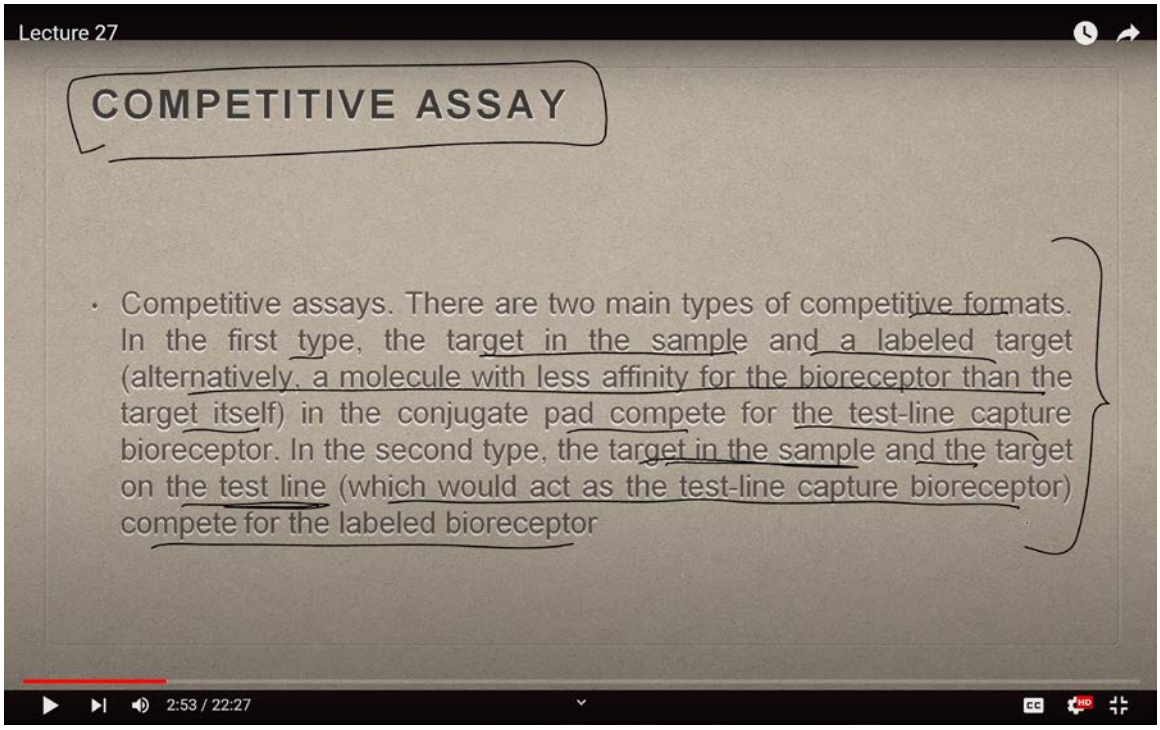

In competitive binding, we introduced the concept of another antigen, one that is similar to the toxin but is a weak binder. This weak binder attaches itself to the surface, albeit not very strongly. When the actual toxin is introduced, it competitively displaces this weak binder, leading to what we refer to as a competitive assay. This concept of competitive assays was the focus of our last class.

We discussed that there are two main types of competitive formats. In the first type, the target in the sample and the labeled target, or a molecule with less affinity for the bioreceptor than the target in the conjugate pad, compete for the test line capture bioreceptors. In the second type, the target in the sample competes with the target on the test line, which serves as the test line capture bioreceptor, for the labeled bioreceptors. This

is the essence of competitive assays, which we covered extensively.

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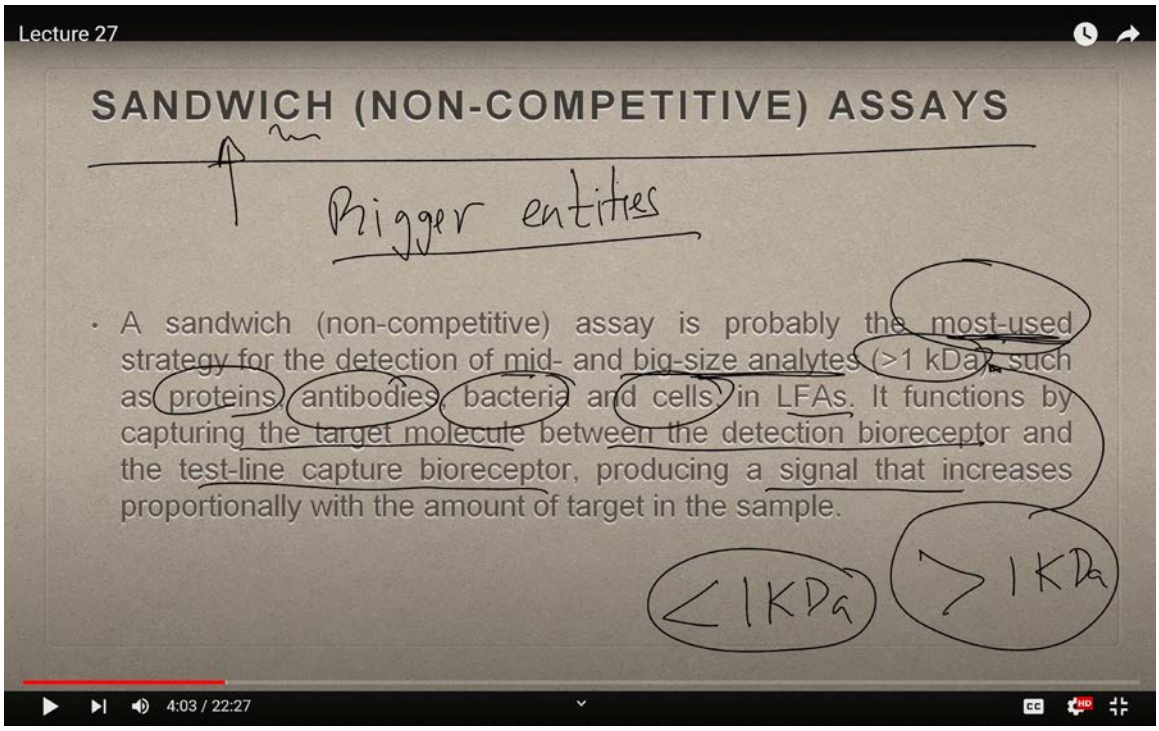

In today's lecture, we will shift our focus from competitive assays to sandwich assays, also known as non-competitive assays. This is the topic for today's class. In today's class, we will explore the sandwich assay, also known as the non-competitive assay, which is arguably the most widely used assay type. This method is particularly popular for detecting mid- to large-size analytes. Unlike the competitive assay, which we previously discussed in relation to molecules smaller than 1 kilodalton, the sandwich assay is designed for molecules greater than 1 kilodalton. Most molecules fall into this category, including proteins, antibodies, bacteria, and cells.

The key point to understand here is that the sandwich assay is specifically tailored for larger entities, such as whole proteins, antibodies, bacteria, and cells in lateral flow assays (LFAs). This assay functions by capturing the large molecule between the detection bioreceptor and the test line capture bioreceptor, resulting in a signal that increases proportionately with the amount of the target molecule in the sample. In other words, the

strength or density of the signal intensifies as more of these molecules are captured.

Now, let's revisit some foundational concepts to put this into perspective. In any assay, the actual signal output could take several forms, such as a fluorescent probe, a color change, or an electrochemical signature. There must be a detectable signal output to confirm the presence of the target. For instance, if there's a change in the signal output, whether it's an increase or decrease in fluorescence, color formation, or electrochemical activity, it indicates that something has bound to that particular site. This is essential for making any scientific claim based on the assay.

To illustrate, when we say the target has reached the binding site and there's a signal output, it means that the target molecule has successfully bound to the detection bioreceptor, triggering a measurable response. This process is central to the operation of these assays.

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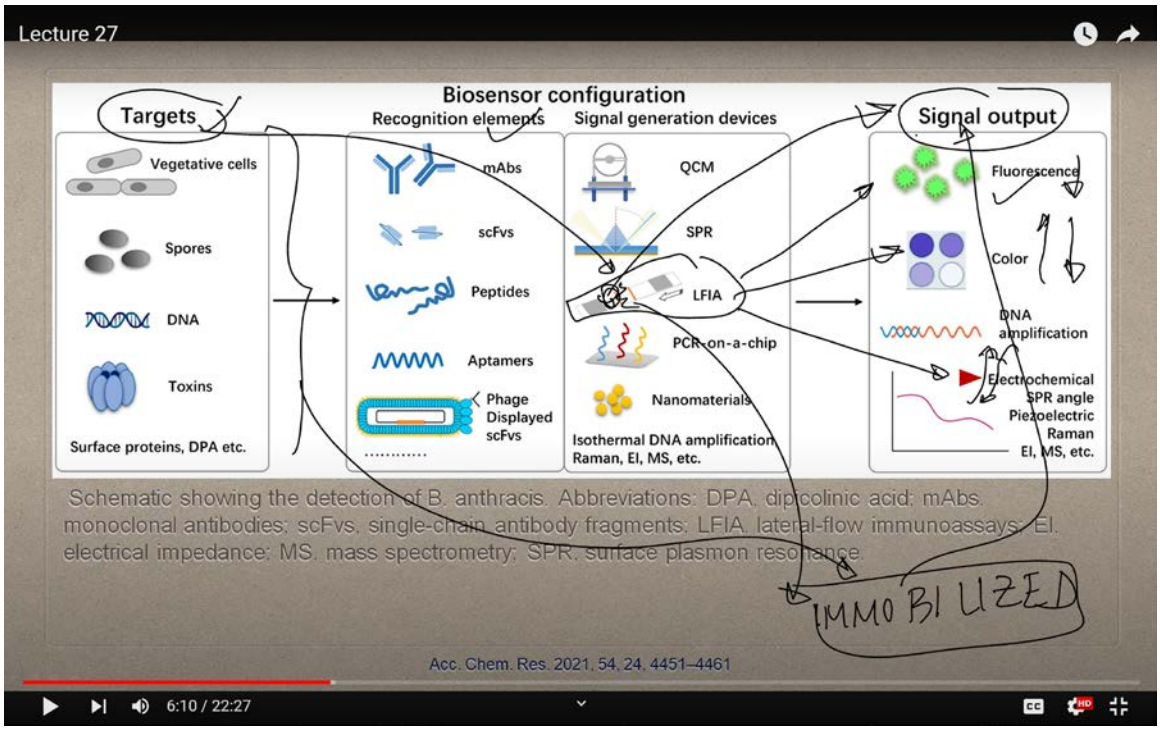

In the context of biosensor configuration, there are four basic elements, with the target recognition element being one of them. In this case, the recognition element is an immobilized antibody. When the target molecule runs across this immobilized surface and

binds to it, a signal is produced. These four elements form the foundation of any biosensor.

Now, moving forward to the concept of larger entities, the non-competitive or sandwich assay requires two receptors that bind to different portions of the target molecule. To put it simply, think of it like getting a second opinion from another doctor to confirm a diagnosis. Just as you would seek multiple confirmations for a diagnosis, the target molecule in a sandwich assay has multiple binding sites.

For example, let's consider molecule A, which has two distinct binding sites, binding site 1 and binding site 2. Now, imagine there's another molecule, B, that is structurally similar to A and also has a binding site similar to one of A's. The assay's effectiveness depends on these multiple binding interactions.

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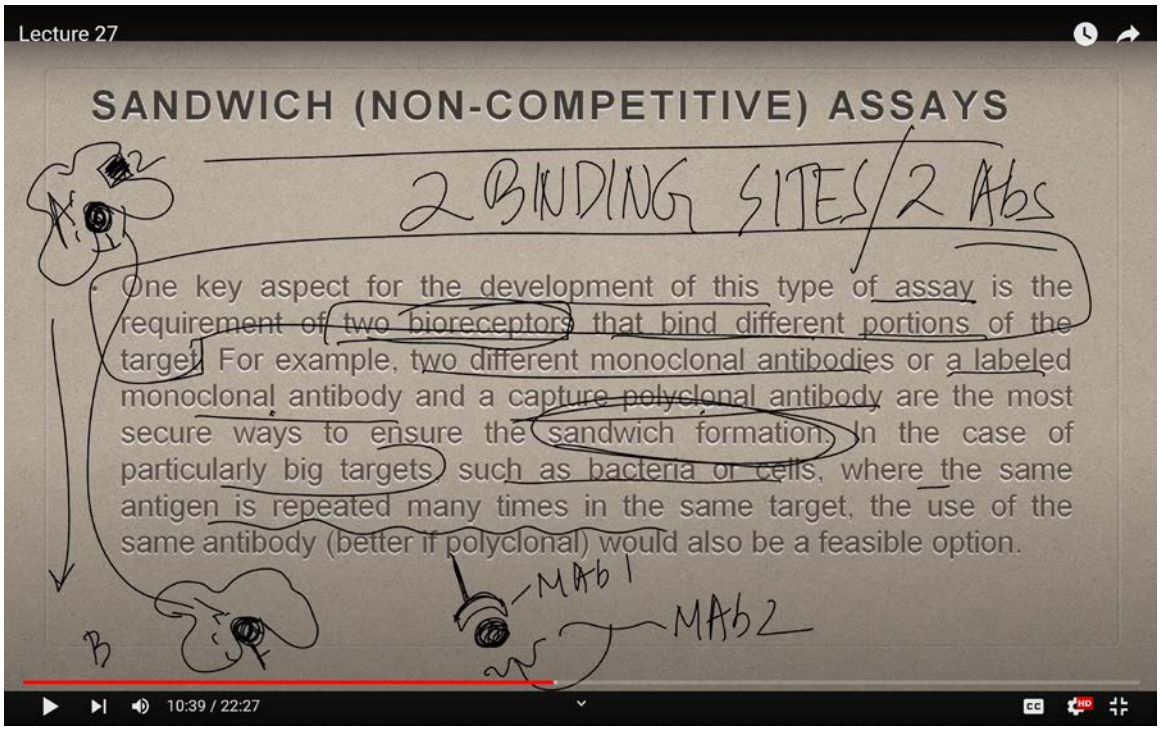

When we observe both sides of this configuration, they appear similar, but let's say our goal is to detect molecule A specifically, without interference from molecule B. If we rely solely on one binding site, there's a risk of false readings because both molecules might bind to that same site. To avoid this, we use two recognition sites. This is why the use of

two different monoclonal antibodies, or a combination of a labeled monoclonal antibody and a capture polyclonal antibody, is crucial.

Let's consider an example with a vegetative cell. To ensure accurate detection, we need the cell to bind to two different kinds of monoclonal antibody fragments, let's call them MAB 1 and MAB 2. MAB 1 might bind to one site on the cell, while MAB 2 binds to another site. This dual binding is essential. On the LFIA plate, we would have both MAB 1 and MAB 2 immobilized. In fact, you can incorporate even more binding sites if you need to confirm beyond doubt that the target is indeed what you're looking for. Given the importance of avoiding false positives, having multiple binding sites is the best strategy.

In essence, the sandwich assay works by capturing the large molecule between the detection bioreceptor and the test line capture bioreceptor, with the signal intensity increasing proportionally to the amount of the target in the sample. The key requirement for this process is having two distinct binding sites.

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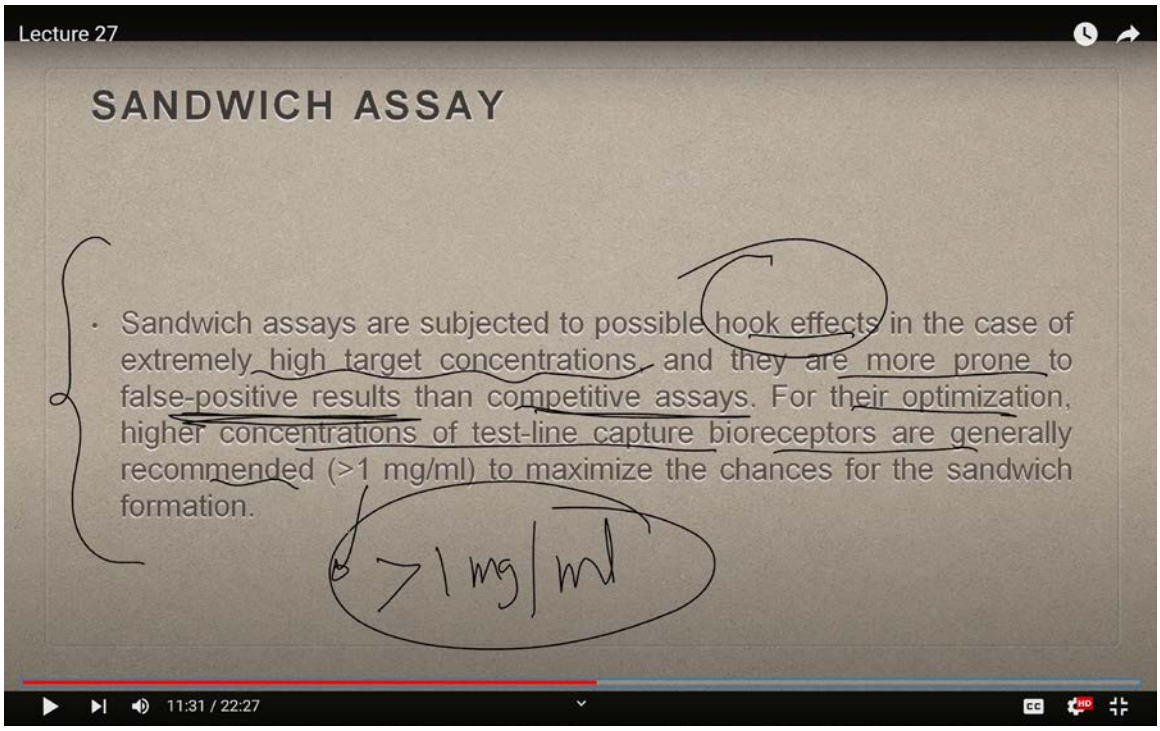

These could be two different monoclonal antibodies, or a combination of a labeled

monoclonal antibody and a capture polyclonal antibody. This approach is the most reliable for ensuring the formation of the sandwich complex, especially when dealing with large targets.

Let’s break it down further. Imagine you have monoclonal antibody 1 on one side and monoclonal antibody 2 on the other, with your target molecule in the middle. The target is effectively “sandwiched” between these two antibodies. This is the fundamental principle of the sandwich assay. In many cases, particularly when dealing with large molecules, this method is highly effective. For example, in the case of bacteria or cells where the same antigen is repeated many times, using the same antibody might be feasible. However, using polyclonal antibodies could provide even better results.

Now, let’s delve a bit deeper into the sandwich assay. It is important to note that sandwich assays can be susceptible to the "hook effect," especially when the target concentration is extremely high. I encourage you to read up on the hook effect, although I will cover it in more detail in a future class. Sandwich assays are also more prone to false-positive results compared to competitive assays. To optimize these assays, it’s generally recommended to use a high concentration of test line capture bioreceptors, typically around 1 milligram per milliliter.

This high concentration means that your requirement for antibodies will be significantly greater than when performing a competitive binding assay. For non-competitive sandwich assays, a denser concentration of antibodies is necessary to maximize the chances of successful sandwich formation. If we look at the schematic representation, you’ll see the sample pad, conjugate pad, test line, control line, and the adsorbent pad on the other side. This is where the binding takes place.

Let's take a look at the schematic of a superparamagnetic lateral flow immunoassay. This is where the retention line comes into play, particularly when using a monoclonal antibody raised against Bacillus anthracis. This assay is specifically designed for detecting Bacillus anthracis spores, and the signal is generated using a magnetic reader. It's called a superparamagnetic lateral flow assay because the binding event alters the paramagnetic signals. As I mentioned, there are multiple ways to detect these signals. You can detect

magnetic signatures, typically using techniques like EPR (electron paramagnetic resonance) or ESR (electron spin resonance). Alternatively, you can use fluorescence assays, colorimetric assays, or other methods, these are just different ways to read the signal.

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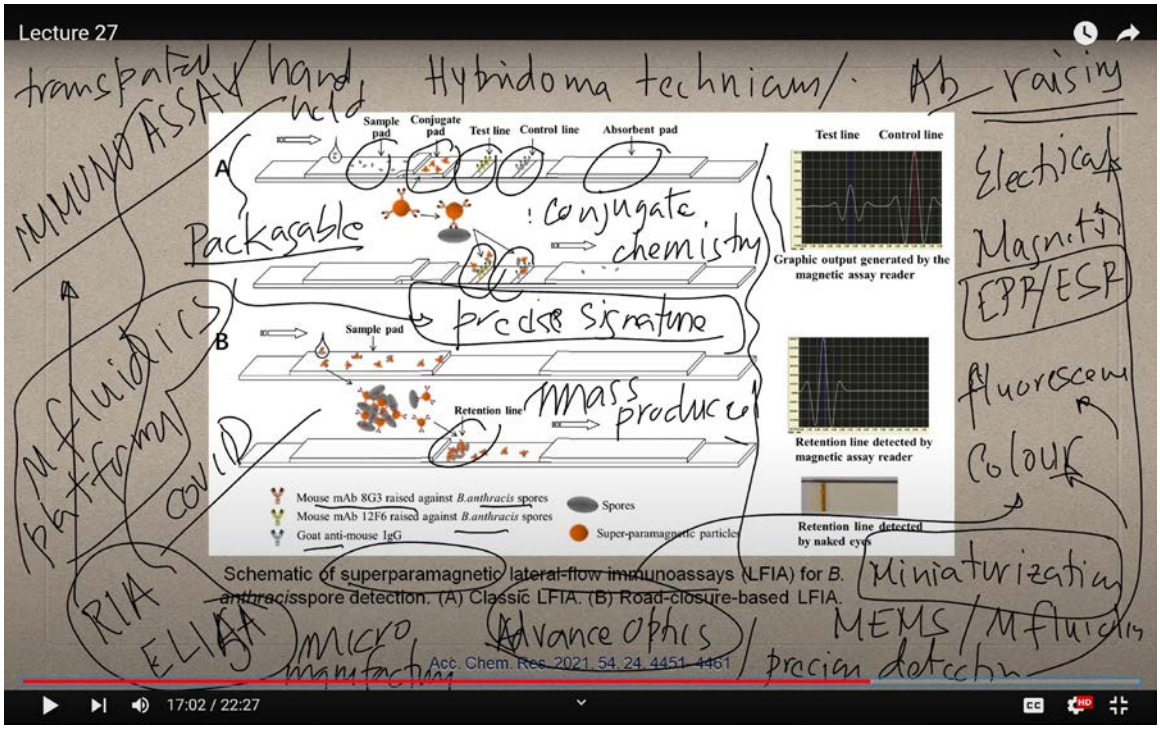

Beyond that, we've discussed various techniques in previous classes, but the fundamental concept remains the same: it's a basic immunoassay technique that has been enhanced through the use of microfluidics platforms. It's important to note that these aren't entirely new techniques; the innovation lies in the miniaturization. Techniques like radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA) have been around for nearly 60 years. However, the real advancements in this field come from miniaturization, the use of MEMS (microelectromechanical systems), microfluidics, and advanced optics. This progress has led to a significant improvement in precision detection, whether it's in terms of electrical, magnetic, fluorescent, or colorimetric signatures. These advancements are further supported by cutting-edge optical technology, hybridoma techniques, and other methods for raising antibodies.

When we look at the broader picture, it’s an integrated approach aimed at developing handheld, miniaturized devices. Some of the essential features of these devices include portability, ease of use, and the ability to be mass-produced. They should also provide precise signatures. If you've seen pregnancy test kits on television or elsewhere, these devices operate on a similar principle. We've seen a significant development of these kinds of assays during the COVID-19 pandemic, where rapid detection was crucial. Much of this progress is driven by micromanufacturing, which plays a critical role in the development of sensory systems.

Another crucial aspect I want to highlight is conjugation chemistry. Conjugation chemistry is vital because it involves linking antibodies to various probes. You might need to conjugate an antibody with a fluorescent probe, an enzyme (as in enzyme-linked immunosorbent assays), a magnetic probe, an electrical probe, or even a dye. This field of conjugation chemistry is vast and highly specialized. As I mentioned in the previous lecture, the entire field of biosensor design requires an integrated approach. To excel in this area, you must first grasp the basic biology and chemistry underlying binding assays.

So, when it comes to detecting a toxin, spore, or pathogen, it's crucial to understand what it binds to. You either need to raise antibodies against it or identify a synthetic target it can bind to, and then you need to develop an assay platform around that. The first step is to determine the level of detection, how low can you go? What is the minimum titer required? When we talk about the minimum titer, we’re referring to the lowest concentration that can be accurately detected.

This brings to mind some early studies, particularly around the year 2000. I vividly remember a significant initiative by the National Institutes of Health (NIH) in the United States. There was immense enthusiasm about the possibility of detecting cancer on a chip. The idea was that any protein or biomolecule generated by the body in response to cancer could be detected with just a tiny sample of blood. Enormous investments were made, but this ambition led to one of the biggest controversies in the field at the time.

The underlying problem was, and still is, that detecting extremely low concentrations of proteins comes with a significant amount of noise. This is a major issue. While you can

detect down to lower levels, it introduces a considerable amount of background noise. For over 25 years, protein assays have faced this challenge, and even now, many of them cannot overcome it due to the noise factor. Despite the promises made and the substantial investments poured into this research, the reality is that detecting very low concentrations of proteins remains a formidable challenge. Sure, many papers have been published on the subject, and I've seen plenty of them, but scaling up these technologies is where they often fall short.

This is one of the critical challenges you must keep in mind: when we discuss the detection of small molecular quantities, every detection element has its associated noise. What we're teaching today may become outdated in a few years. As we continue to innovate, new methods of noise reduction are essential, and this is the ongoing challenge. Many have worked on these problems for decades, but the question remains, how low can we go, and what will be our ultimate detection limit?

Remember, there's vast potential for research and career development in these areas, as long as you grasp the fundamental principles and explore innovative ways to minimize noise. So, I will close in here. In the next class, we will move on to the botulinum toxin. Thank you.