Design for Biosecurity

Prof. Mainak Das

Department of Design

Indian Institute of Technology, Kanpur

Lecture 26

Design and Fabrication of Lateral-Flow Immunoassays

Welcome back to our lecture series on biosecurity measures, with a focus on the development of biosensors. In our previous session, we explored the production of antibodies using various techniques, including hybridoma technology, and delved deeply into the molecular methods employed in antibody generation.

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These antibodies are undeniably among the most powerful tools available for biosensor development. I emphasized the importance of future designers in this field possessing a robust understanding of biology, chemical engineering, physics, and engineering disciplines. This also includes expertise in micromachining, microfabrication, microfluidics, microelectromechanical systems (MEMS), packaging, and the integration of these elements using advanced software tools.

Today, we will discuss a significant breakthrough in this realm: lateral flow immunoassays (LFIA). As illustrated in the accompanying image, LFIA is highlighted within a specific context.

Lateral flow immunoassays have revolutionized the creation of detection tools, employing various base materials that have become increasingly flexible compared to the more rigid matrices used initially. Before we dive into the specifics of LFIA, it’s crucial to acknowledge the monumental advancements over the past 20 to 30 years in micromachining, microfluidics, and BioMEMS.

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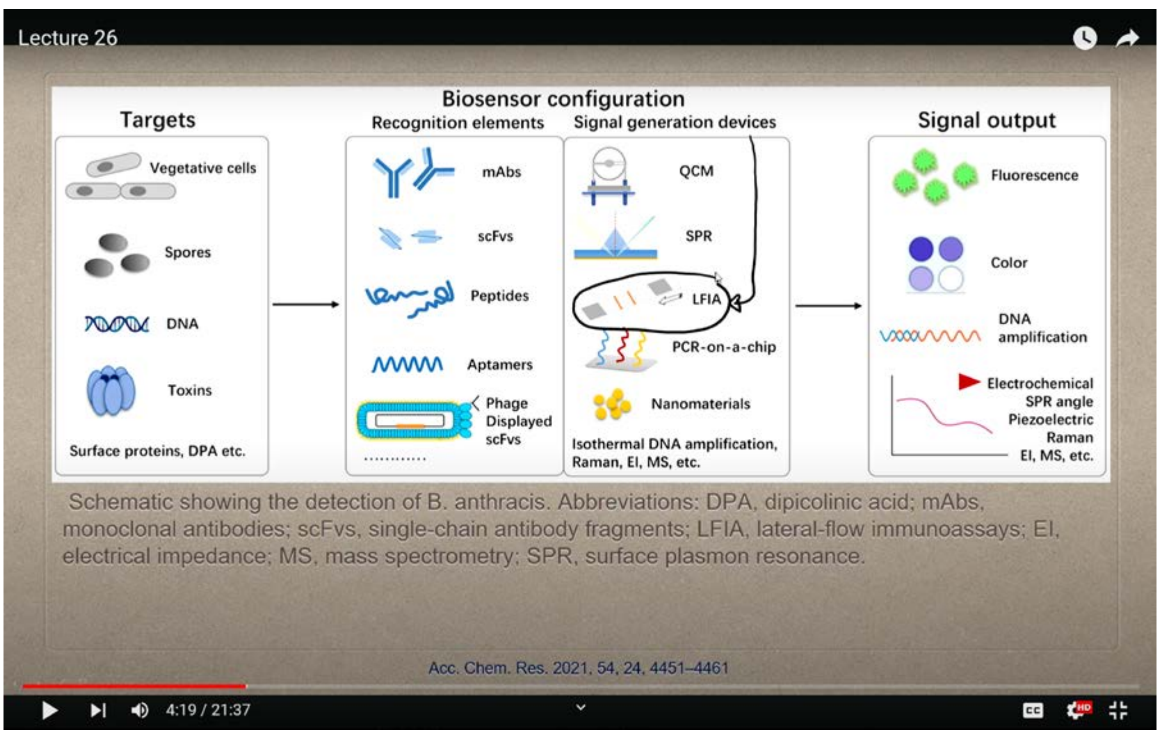

These three areas have enabled the fabrication of intricate microstructures on diverse substrates, which have been instrumental in developing flow cells and microchannels. With the maturity of micromachining technology, we now have the capability to integrate numerous optical devices within these micromachined structures. This fusion of microelectronics, micromachining, and microfabrication has utterly transformed the field of biosensor development. Despite these technological strides, the underlying principle remains consistent: the necessity of an antibody-antigen interaction, where an antibody targets a specific toxin and binds to it.

However, as I have previously emphasized, the primary challenge lies in differentiating between noise and signal, especially since we are dealing with molecular interactions. Techniques such as conjugated secondary antibodies, fluorescent probes, and nanoprobes have been pivotal in enhancing signal detection. The advancements in microfluidics, microfabrication, and BioMEMS have played a crucial role in reaching the current state of technology.

Today, we will be focusing on signal generation devices, particularly lateral flow immunoassays (LFIA), which stand as a testament to the progress in this field.

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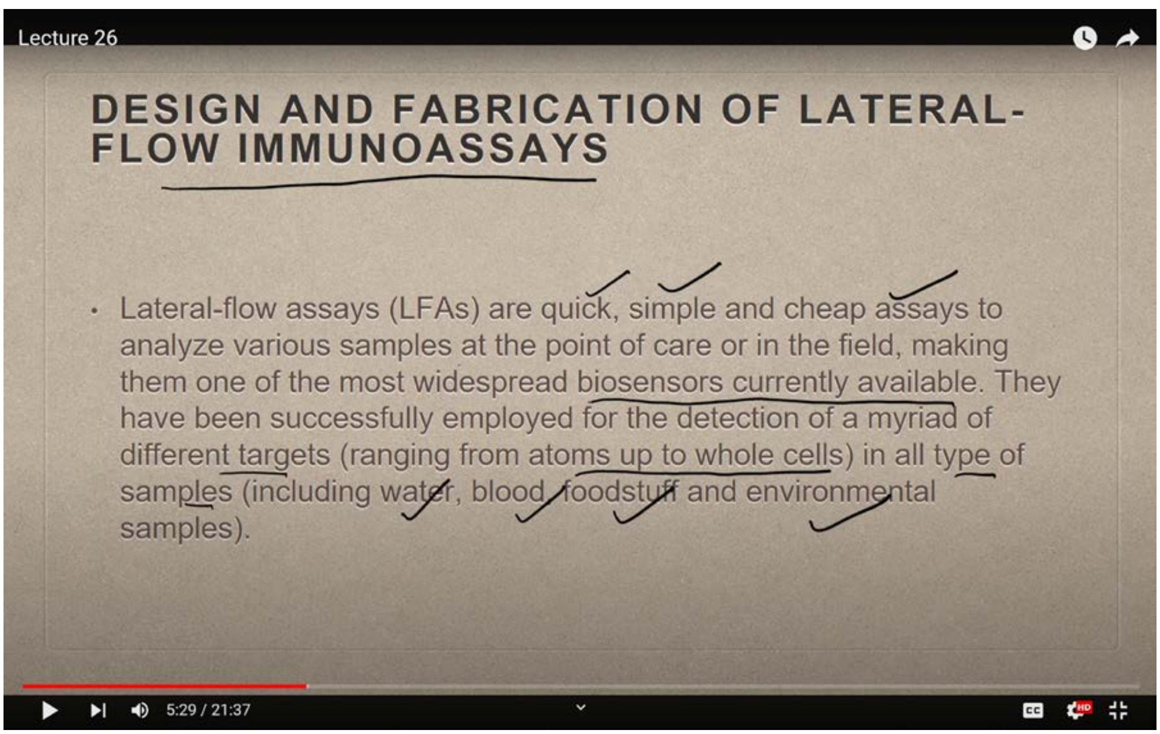

As the name suggests, a lateral flow immunoassay (LFIA) is fundamentally an immunoassay, meaning it relies on antigen-antibody reactions. Today's lecture will focus on the design and fabrication of these LFIAs. These assays are renowned for being quick, simple, and cost-effective, making them indispensable tools for analyzing a wide range of samples at the point of care or in the field. Due to their versatility and ease of use, LFIAs are among the most widespread biosensors available today. They have been successfully employed in the detection of an extensive array of targets, ranging from individual atoms to entire cells.

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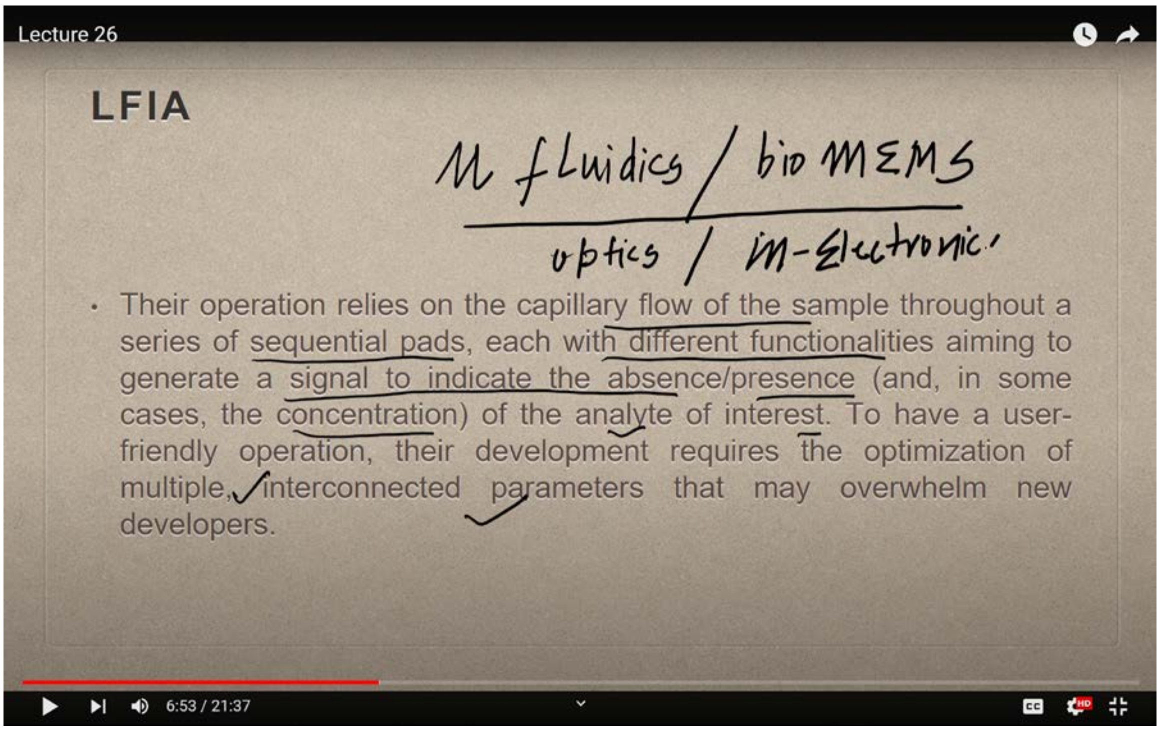

LFIAs can be utilized with a variety of samples, including water, blood, food products, environmental samples, urine, and even feces. Their practicality makes them an invaluable tool for field applications, as they can be easily transported over long distances and used for routine, as well as highly complex, detections.

The operation of an LFIA is based on the capillary action of samples as they move through a series of sequential pads, each designed with specific functionalities. The ultimate goal is to generate a signal that indicates the presence or absence of the target analyte, and in some cases, even measure its concentration. Developing user-friendly LFIAs requires the optimization of multiple interconnected parameters, which can be overwhelming for new developers.

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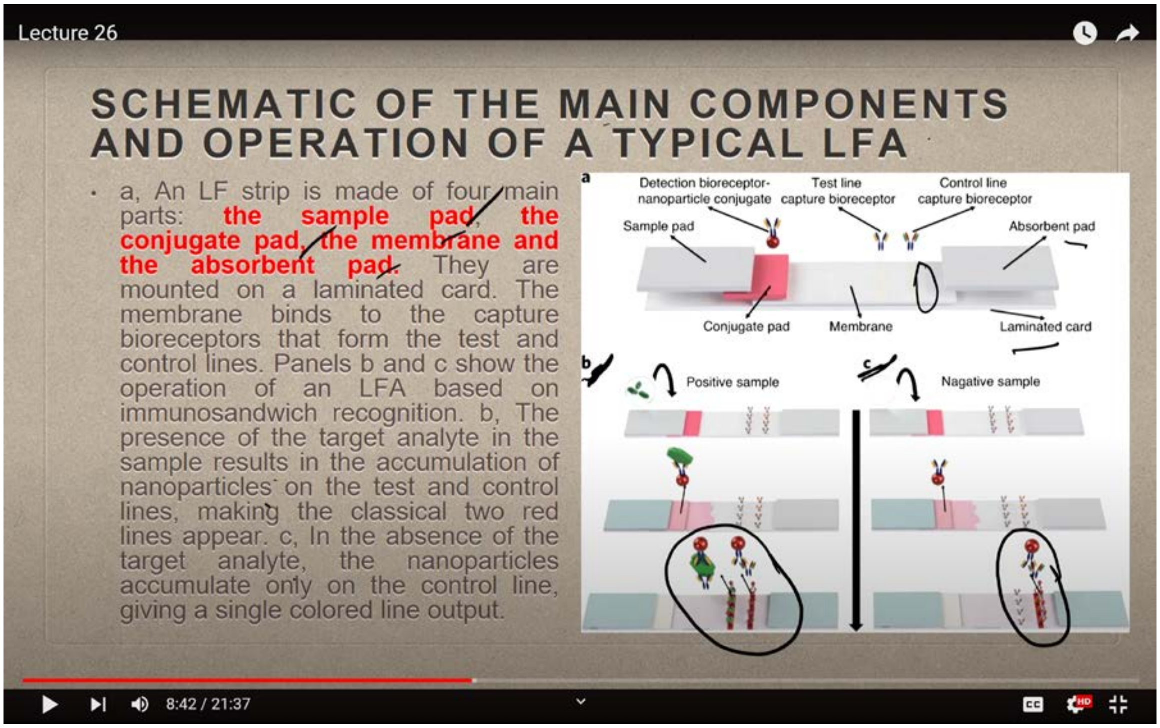

As mentioned earlier, the development of LFIAs poses challenges in microfluidics, biomes, optics, and microelectronics, as many assays depend heavily on the engineering of these devices. To better understand this, let's examine the schematic of a typical LFIA strip, which consists of four main components: the sample pad, the conjugate pad, the membrane, and the absorbent pad. These components are all mounted on a laminated card. The membrane plays a crucial role by binding to the capture receptors that form the test and control lines.

In this setup, the sample pad is where the sample is introduced. The conjugate pad contains the bioreceptor-nanoparticle conjugates, which are responsible for detection. The test line on the membrane captures the bioreceptor, while the control line ensures the assay is functioning correctly. Although the adsorbent pad and laminated card are visible, the detection units themselves are not immediately apparent.

In panels B and C, you can observe the operation of an LFIA based on immune sandwich recognition. When the target analyte is present in the sample, nanoparticles accumulate on both the test and control lines, resulting in the appearance of the characteristic two red lines, as shown. In the absence of the target analyte, nanoparticles accumulate only on the control line, producing a single red line.

This represents a typical, straightforward version of an LFIA assay. However, consider the scenario where these analytes must move along the strip. The more the assay is miniaturized, the greater the role microfabrication plays in its operation. The assay essentially functions like a flow cell, where the analyte binds to specific binders on an adsorbent surface.

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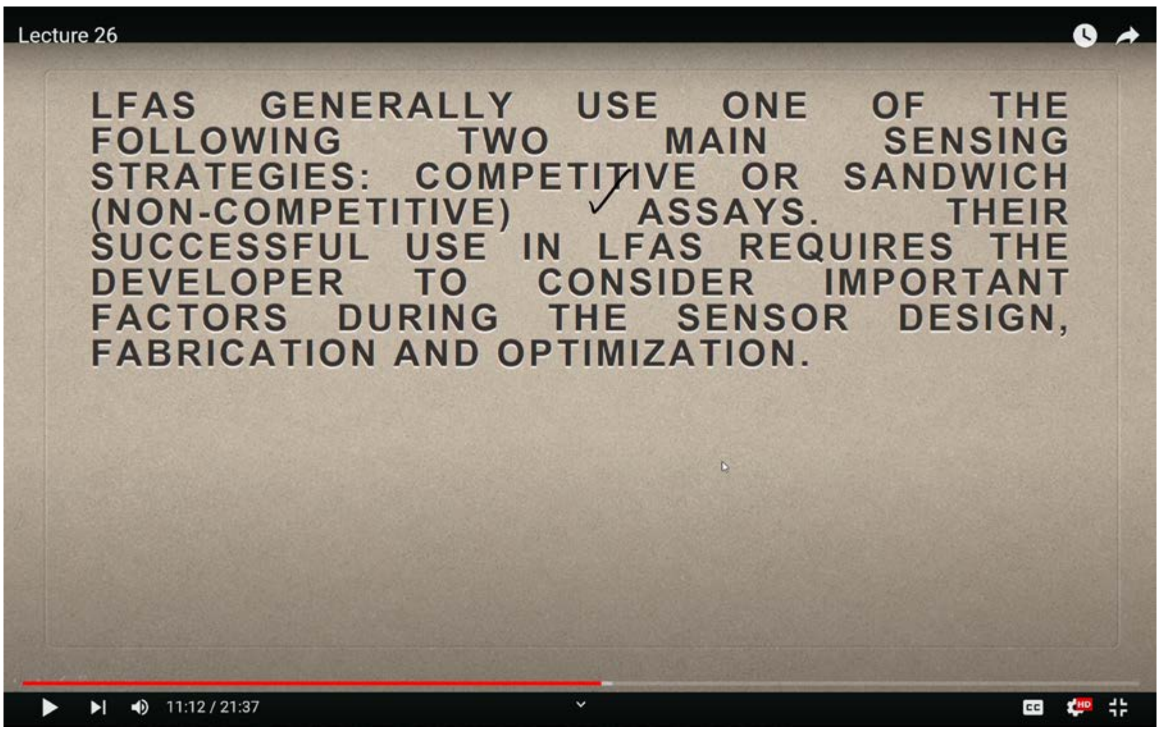

In essence, the process involves a surface coated with highly specific binders. When a sample is introduced, it flows across this surface. If the sample contains an antigen that matches the antibody on the surface, binding occurs. The speed at which this binding happens can be crucial, as the bound antibody typically carries a marker, this could be a fluorescent marker, a dye, or another type of signal, that indicates the binding event.

Once the binding event occurs, it is crucial to capture the resulting signal. However, for the assay to work effectively, you must ensure that the sample flows uniformly over the surface where the antibodies are present. This reaction needs to happen in a highly controlled and precise manner. When developing biochips or biosensors, particularly for commercial use, the level of precision required is immense. This precision is essential because any commercial product must exhibit consistency, anomalies between products are unacceptable. Variations between two sensors from the same company can lead to significant issues for the end-user. Therefore, each sensor must be meticulously measured and manufactured to eliminate any ambiguity.

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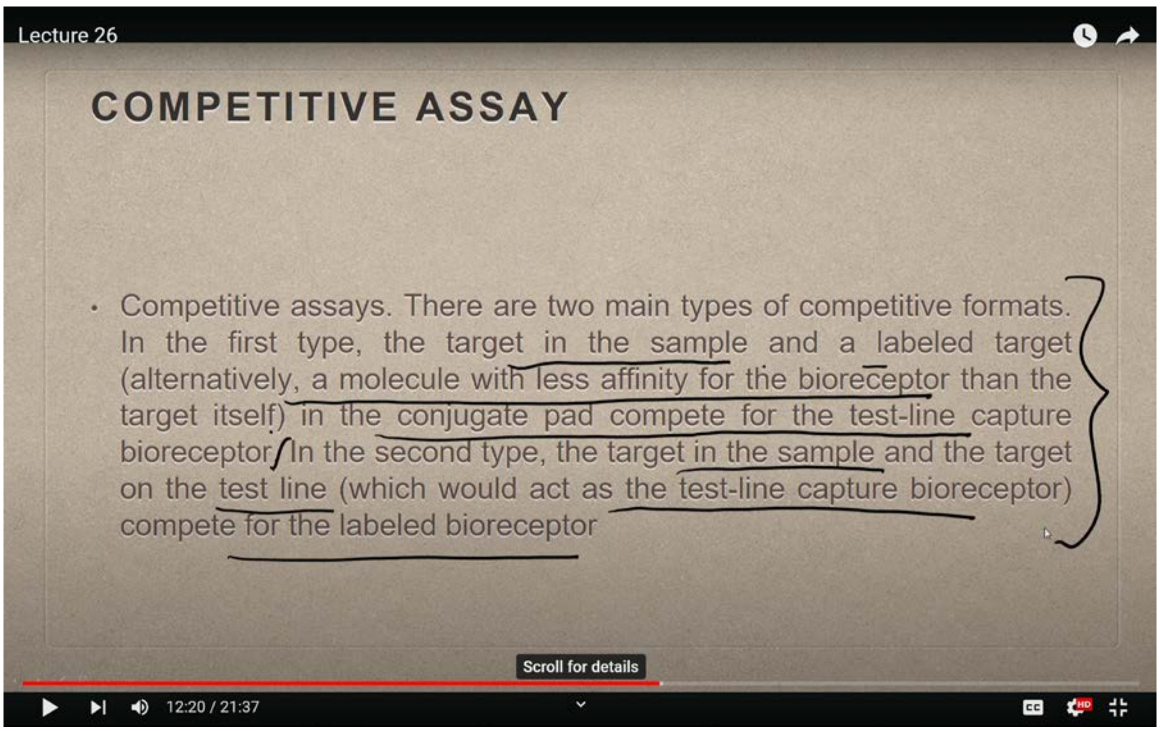

Now, when it comes to sensing strategies in LFIAs, two primary approaches are generally employed: competitive binding assays and sandwich or non-competitive assays. The successful implementation of these assays in LFAs requires careful consideration of several critical factors during the design, fabrication, and optimization phases of sensor development.

There are multiple ways in which binding can occur, but let's focus on the competitive and non-competitive binding assays. In competitive assays, there are two main competitive formats to consider. In the first format, the target molecule in the sample competes with a labeled target, or alternatively, with a molecule that has a lower affinity for the bioreceptor than the target itself, for capture by the bioreceptor on the test line.

In the second format, the target molecule in the sample competes with the target molecule already present on the test line, which acts as the test line capture bioreceptor. This is essentially the reverse format of the first type, and different types of competitive assays can be employed depending on the specific application. The key principle of a competitive binding assay is that the target molecule competes more effectively than a weaker binding molecule.

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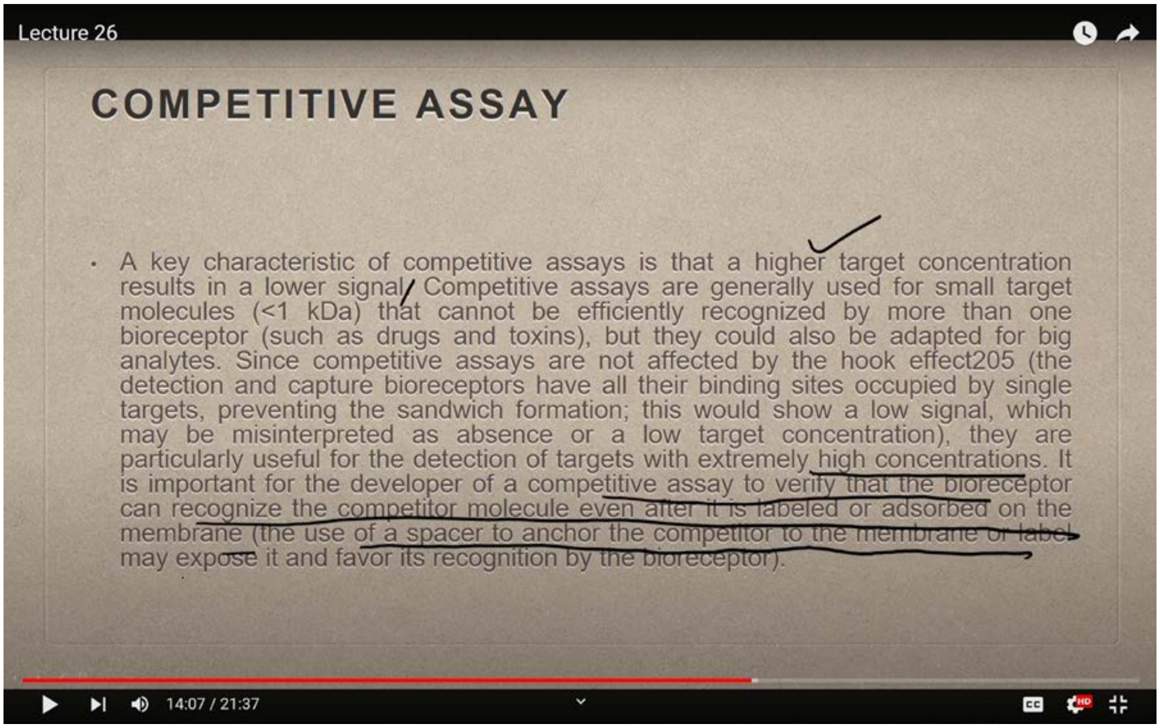

The outcome of these binding assays helps determine the concentration of the molecule of interest or pathogen present in the sample. A crucial characteristic of competitive assays is that a higher concentration of the target molecule results in a lower signal. Competitive assays are typically used for small target molecules, those less than one kilodalton in size, that cannot be efficiently recognized by more than one bioreceptor, such as drugs or toxins. However, they can also be adapted for larger analytes.

One significant advantage of competitive assays is that they are not affected by the hook effect. The hook effect occurs when all binding sites on the detection and capture bioreceptors are occupied by a single target, preventing the formation of a sandwich complex, which would otherwise result in a low signal. This low signal could be mistakenly interpreted as a low target concentration or even the absence of the target. Competitive assays are particularly valuable for detecting targets present at extremely high concentrations.

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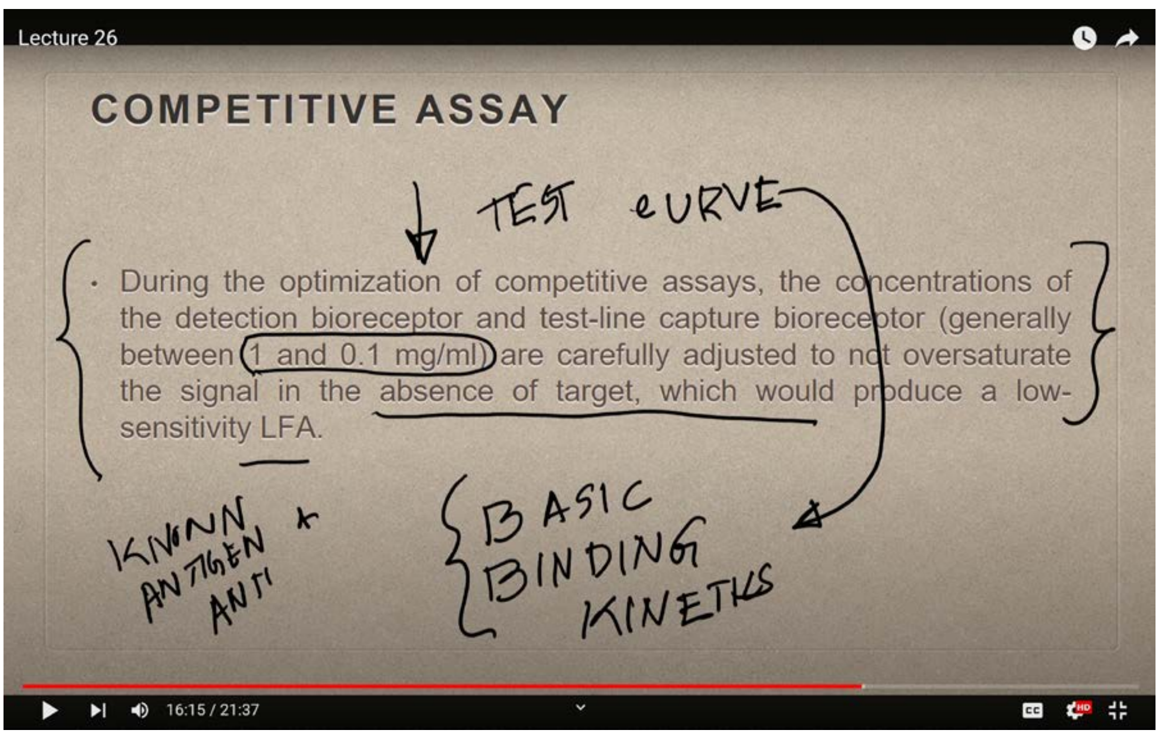

For a competitive assay to be effective, the developer must ensure that the bioreceptor can still recognize the competitor molecule after it has been labeled or adsorbed onto the membrane. Using a spacer to anchor the competitor to the membrane or label may help expose it and improve its recognition by the receptors. This is the foundational principle behind competitive assays.

When optimizing competitive assays, it's crucial to carefully adjust the concentrations of both the detection bioreceptor and the test line capture bioreceptor, typically within the range of 1 to 0.1 milligrams per milliliter. This fine-tuning is essential to avoid oversaturating the signal in the absence of a target, which would result in a low-sensitivity lateral flow assay (LFA). It's important to remember that every system has a detection limit. Exceeding this limit by oversaturating can lead to ambiguous results, causing confusion and inaccuracies. Therefore, it's vital to ensure that the binding concentrations used are within the permissible limits of the assay.

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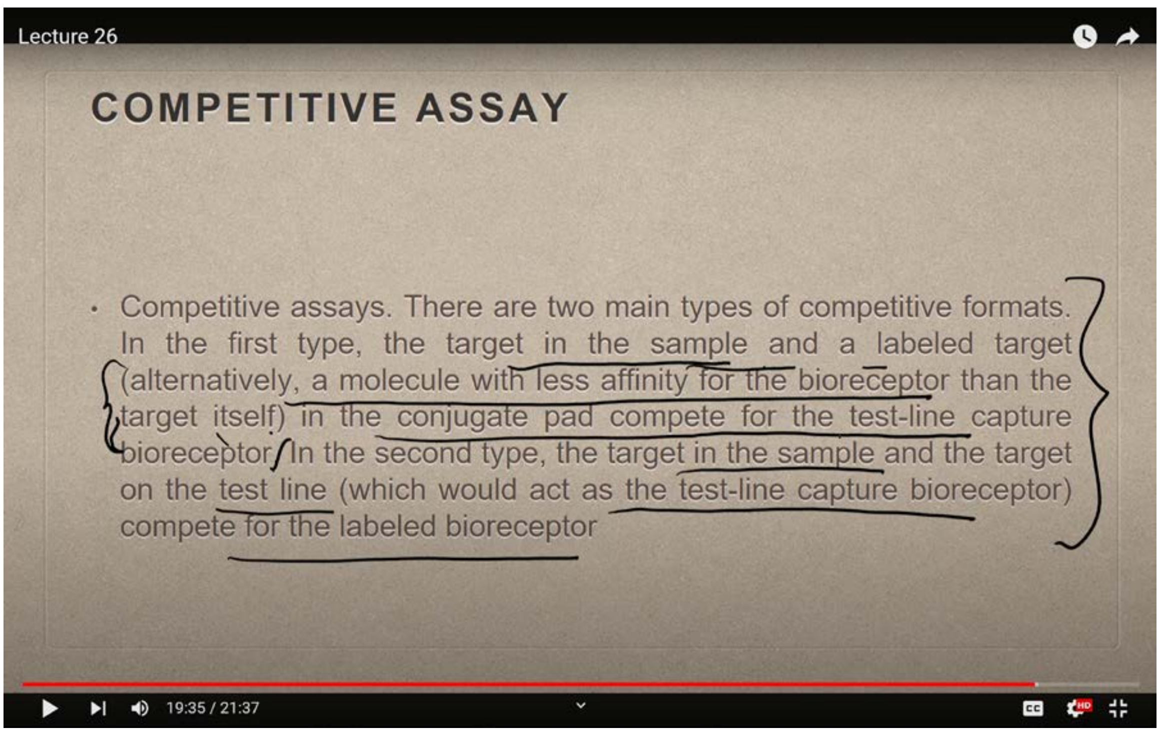

To achieve optimal results, the development of initial test curves is necessary. These test curves help establish the basic binding kinetics, making it easier to detect and interpret the results accurately. Without these foundational test curves, detecting and analyzing the target becomes significantly more challenging. It's also important to have a clear understanding of known antigen-antibody reactions. Working with such systems without precise knowledge of the concentrations involved can be quite difficult.

Now, revisiting the fundamentals of competitive assays, we recognize that there are two primary types of competitive formats. In the first type, the target in the sample and a labeled target, typically a molecule with lower affinity for the bioreceptor than the actual target, compete for the test line capture receptors located on the conjugate pad. To illustrate this, imagine that you have an antibody with a weak binder attached to it, which is not the target molecule. This weak binder is loosely attached but still binds to the surface.

However, when a stronger competitor, a molecule that can bind more robustly, comes along, it will displace the weaker binder and attach itself more firmly to the surface. This scenario exemplifies a competitive assay, where there is an active competition between the antigen or toxin and another agent on the test line. The test line already contains a weaker binder, and the goal is to push the target antigen or toxin to bind more effectively by outcompeting the weaker binder. This competition is key to the functionality of the assay.

To summarize, in the first type of competitive assay, the target in the sample and the labeled target, or a molecule with less affinity for the bioreceptor than the target itself, compete for binding. This competition is a critical aspect of the assay's design and functionality.

When a molecule with lesser affinity is bound to a site and one with higher affinity comes along, it will displace the weaker molecule and take its place. Does that make sense? This concept is crucial for understanding what’s happening at the molecular level. Essentially, on the test line, there are already binders, these could be antibodies, for example, attached to weakly binding antigens.

Now, when the sample flows through, these weakly bound antigens are toppled by the stronger, more specific antigens in the sample, which then bind more tightly to the site. Imagine it like people holding hands with a loose grip; when someone with a stronger grip comes along, they replace the weaker handhold. This replacement process is what we refer to as competition, there's a competition between molecules to bind to the target. This principle is the foundation of competitive assays in lateral flow immunoassays (LFIAs).

Looking at the slide again, in the first type of competitive assay, the target in the sample and a labeled target, or a molecule with less affinity for the bioreceptor than the actual target, compete for the test line capture bioreceptor located on the conjugate pad. 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 receptors.

In the provided image, this is your positive sample, and you can see the test lines. On the test line, competitive binding occurs. This competitive binding is crucial because it helps rule out the possibility of nonspecific binding. What happens during competitive binding is that the weaker binders are dislodged and move further along.

This is why having both a positive and a negative control in these tests is so important, it helps provide a clear understanding of how these binding assays are evaluated and quantified, without running into the issue of oversaturation, which must be avoided at all costs. Thank you, and we'll move on to the next class after this.