LATERAL FLOW ASSAY BASED POINT OF CARE DIAGNOSTIC KIT

Aim:

To understand the function of Lateral Flow Assay by using diagnostic kit and to analyze the absorption spectrum of gold nanoparticle conjugates at the control line of a lateral flow assay-based pregnancy strip.

Apparatus:

- Lateral flow assay-based pregnancy strip
- Spectrometer
- Computer (OceanView Software)

Objective:

To analyse the spectrum characteristics of the conjugates of gold nanoparticles on the control line to confirm that the assay is capable of identifying human chorionic gonadotropin (hCG) in urine samples.

Background:

Lateral Flow Assay (LFA) technology, also referred to as lateral flow immunoassay or immunochromatographic assay, is a widely utilized diagnostic approach known for its simplicity, speed, and user-friendliness. Initially conceived in the late 20th century, LFAs have become indispensable across diverse domains such as medical diagnostics, environmental monitoring, and food safety testing. The key strengths of LFAs include their swift results, straightforward operation, and minimal equipment demands, rendering them especially suitable for point-of-care diagnostics and field applications. LFAs have played a pivotal role in transforming diagnostic testing, providing a portable and effective solution for a variety of healthcare and non- healthcare scenarios. Their versatility and adaptability have spurred the creation of LFAs capable of detecting an extensive array of substances, ranging from pathogens and antibodies to hormones and environmental pollutants.

Working principle:

The working principle of a lateral flow assay (LFA) is based on the capillary action that draws a liquid sample along a test strip containing specific reagents. When the sample is applied to the sample pad, it migrates along the strip by capillary action, passing through the conjugate pad, nitrocellulose membrane, and absorbent pad.

The key components of an LFA are:

1. **Sample pad**: Absorbs the sample and controls its flow onto the conjugate pad.

2. **Conjugate pad**: Contains colored particles (e.g., colloidal gold) conjugated with antibodies specific to the target analyte. When the sample reaches this pad, the analyte binds to the conjugated antibodies.

3. **Nitrocellulose membrane**: Provides the solid support for immobilizing test and control line reagents. As the sample moves along the membrane, the analyte- antibody complexes bind to the test line, forming a colored band. The intensity of the test line is proportional to the analyte concentration.

4. **Absorbent pad**: Draws the sample through the membrane by capillary action and prevents backflow.

The versatility of LFA technology makes it applicable to a wide variety of diagnostic applications, including infectious disease diagnosis (COVID 19), Family planning (e.g., pregnancy kits). The LFA consists of nitrocellulose paper which is housed inside a plastic casing. Therefore, the LFA works similar to paper chromatography, in which the sample extracted buffer serves as a mobile phase while the nitrocellulose paper acts as a stationary phase. Taking the case of pregnancy kits, human chorionic gonadotropin (hCG) protein is used to detect using these kits.Pregnancy kits detect human chorionic gonadotropin (hCG) protein using LFAs. The process involves:

- **Sample Application:** The sample solution is dropped onto the sample pad, releasing gold nanoparticle anti-hCG antibody conjugates from the conjugate pad. If the sample contains hCG, it binds selectively to the gold nanoparticle antibody conjugates.
- **Test Line:** The test line contains nitrocellulose immobilized polyclonal anti-hCG antibodies. These antibodies bind to the hCG-bound anti-hCG antibody gold nanoparticle conjugate, forming a sandwich complex. The presence of gold nanoparticles on the test line appears red on the macroscopic scale. Gold nanoparticle anti-hCG antibody conjugates with no hCG passes to the control line.
- **Control Line:** The control line antibody is specific for the antibody attached to the AuNP. The AuNP-conjugate always binds to the control line antibody, producing a visual signal in a successful LFA.

The sample pad, conjugate pad, membrane, and absorbent pad, all affixed to a laminated card. The membrane attaches to capture bioreceptors forming the test and control lines. Illustrations in panels b and c elucidate the functioning of an LFA based on immunosandwich recognition.

Principle of Operation - Immunosandwich Recognition:

- Target Analyte Presence: If the target analyte is present in the sample, nanoparticles accumulate on both the test and control lines due to immunosandwich recognition, resulting in the appearance of two red lines.
- Absence of Target Analyte: In the absence of the target analyte, nanoparticles only accumulate on the control line, leading to the appearance of a single colored line output.

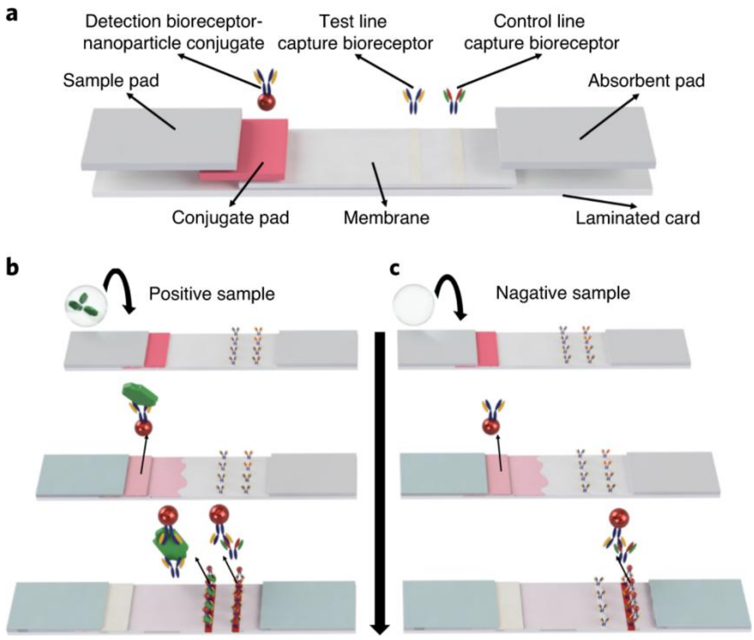

Figure 1: An LFA strip

Output Interpretation:

- Two Red Lines: Indicates a positive result, confirming the presence of the target analyte in the sample.
- Single Colored Line: Indicates a negative result, suggesting the absence of the target analyte in the sample.

Procedure:

1. Drop 2-3 drops of urine (here we will use water) to the dedicated slot in sample pad.
2. Due to capillary action the fluid flows through the membrane crossing the conjugate pad towards absorbent bed.
3. After 5-10 seconds, the results can be seen. Here, one red line will appear at the control line since no biorecognition activity happens on the surface of the gold nanoparticle.
4. Using a reflection probe connected to a light source and a spectrometer, the absorption spectrum of the conjugate at the control line can be seen.

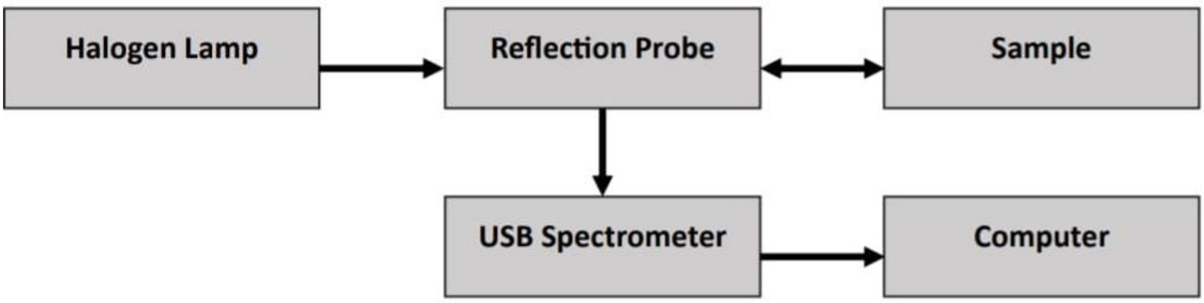

Figure 2: Experiment Block Diagram.

Results:

Plot the absorption spectrum for the gold nanoparticle conjugate as seen in the control line of the pregnancy strip.

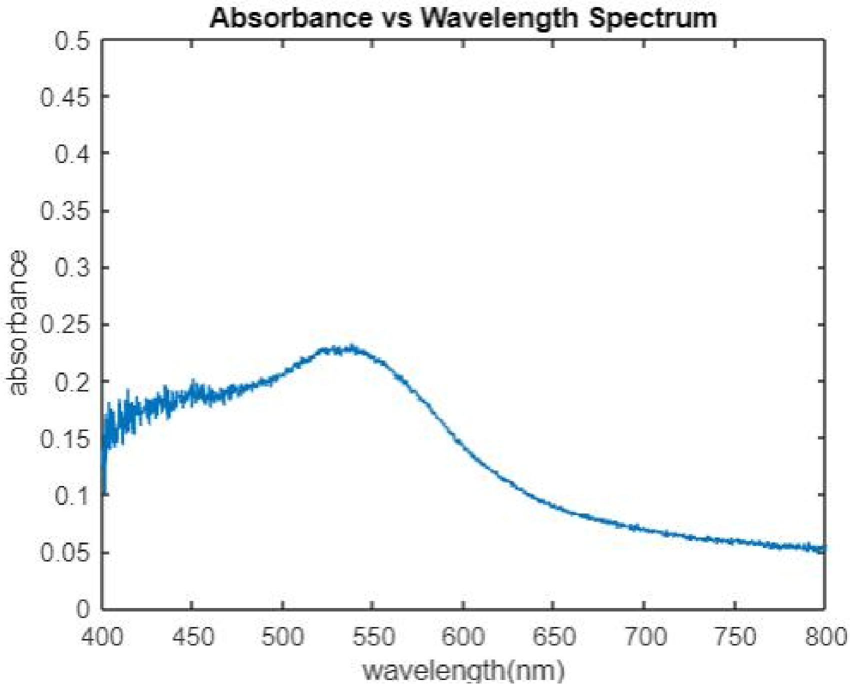

Figure 3: Absorption spectrum for the gold nanoparticle conjugate

Absorbance vs. wavelength plots in lateral flow assays show how gold nanoparticles, linked to antibodies, interact with antigens in patient samples. The peak is around 520nm wavelength. The spectrometer exhibits high absorbance around this wavelength.