Online Training Curriculum

Confirmation Methods for Food contaminants

Principles of Lateral Flow Devices

Introduction to LFD techniques

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Overview

The most used immunoassays tests for the detection/identification of food contaminants are ELISA and lateral flow devices. ELISA tests are usually performed in microtiter plates but can be used in other formats. Lateral flow tests or “dipsticks” employ the same immunoassay principles as the ELISA tests but coat the antibodies and other reagents on a nitrocellulose membrane rather than the inside of test wells and they use different conjugates to generate signal rather than enzymes bead conjugates [1].

Principle

The underlying principle of an LFD is relatively simple: a liquid sample containing the targeted analyte moved via the paper-enabled capillary action through various zones within a paper strip. During the transport, the analyte interacts with antibodies that have been pre-coated onto the strip and consequently gets captured at the detection sites (test line and control line). The results are usually obtained within 5-20 minutes. The development of a color at the test line indicates the presence of the analyte, while a colorimetric response at the control line indicates that sample flow has correctly occurred through the strip and that the device has worked correctly.
LFDs use immunoassay technology using nitrocellulose membrane, coloured nanoparticles (or labels), and typically antibodies, to produce results. It consists of four parts: a sample pad, a conjugate pad, a porous membrane, and an absorbent pad (see **figure 1 below**).

- The sample pad acts as the first stage of the absorption process. Sometimes, this pad contains a filter, to ensure the accurate and controlled flow of the sample.
- The conjugate pad is the part containing the coated conjugated labels and antibodies. If the target is present, the immobilised conjugated antibodies and labels will bind to the target and continue to migrate along the test.
- As the sample moves along the device the binding reagents situated on the nitrocellulose membrane will bind to the target at the test line. If the analyte is present, a coloration will be developed, and the density of the color will vary depending on the quantity of the target present. Some food contaminants may require quantification and this is where a rapid test can be combined with a reader to provide quantitative results.

- The sample will pass through the nitrocellulose membrane into the absorbent pad. The absorbent pad will absorb the excess volume of the sample. The specification of the absorbent pad will have an impact on the volume of sample the test can incorporate.

While not strictly necessary, most tests incorporate a second line which contains an antibody that picks up free conjugate molecules in order to confirm the test has operated correctly. Regardless of the quantity of analyte in the sample, an anti-species antibody at the control line will bind the nanoparticle, yielding a strong control line signal that demonstrates that the assay is functioning correctly.

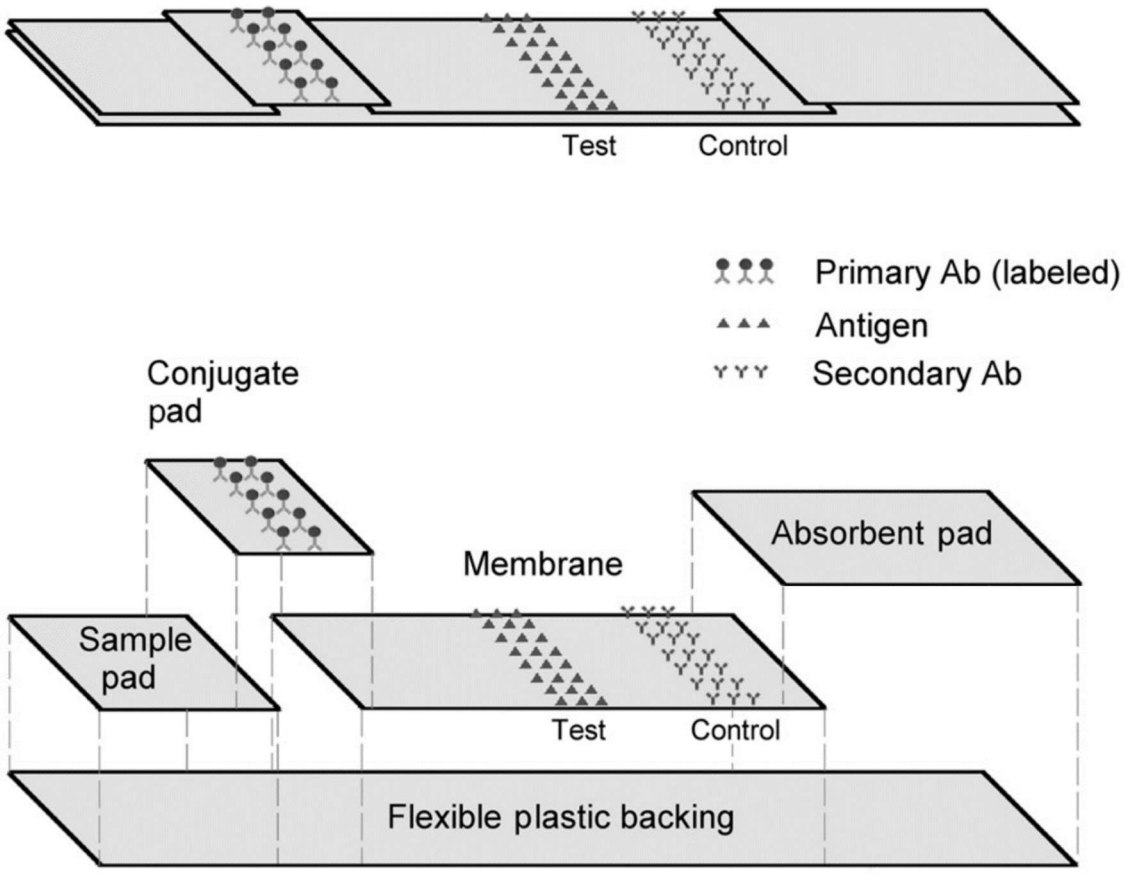

Figure 1: Components of a generic lateral flow device. Sample extract is added to the sample pad and flows, through capillary action, through the conjugate pad and membrane towards the absorbent pad. The presence of analyte in the sample inhibits the binding of conjugate to the ‘test’ (immobilized analyte) line, but does not inhibit the binding of conjugate to the ‘control’(anti-species antibody) line [2].

Types of LFDs

LFDs can be developed to be used in a dipstick format or in a housed cassette. Both will work in a similar way, it is just dependent on the manufacturer, sample matrix, and the market requirement, as to which format is suitable. Using names such as rapid or quick test can lead to misunderstanding about capabilities of LFDs. However, LFDs are compact, easy-to-use, and offer a great flexibility.

Sandwich assays

A positive test is represented by the presence of a coloured line at the test line position. The sandwich assay format is typically used for detecting larger analytes that have at least two binding sites, or epitopes

Competitive assays

A positive test is represented by the absence of a coloured line at the test line position. Competitive assays are generally used for smaller analytes since smaller analytes have fewer binding sites. In this format, the test line typically contains the analyte molecule, usually a protein-analyte complex, and the conjugate pad contains the detection antibody-nanoparticle conjugate.

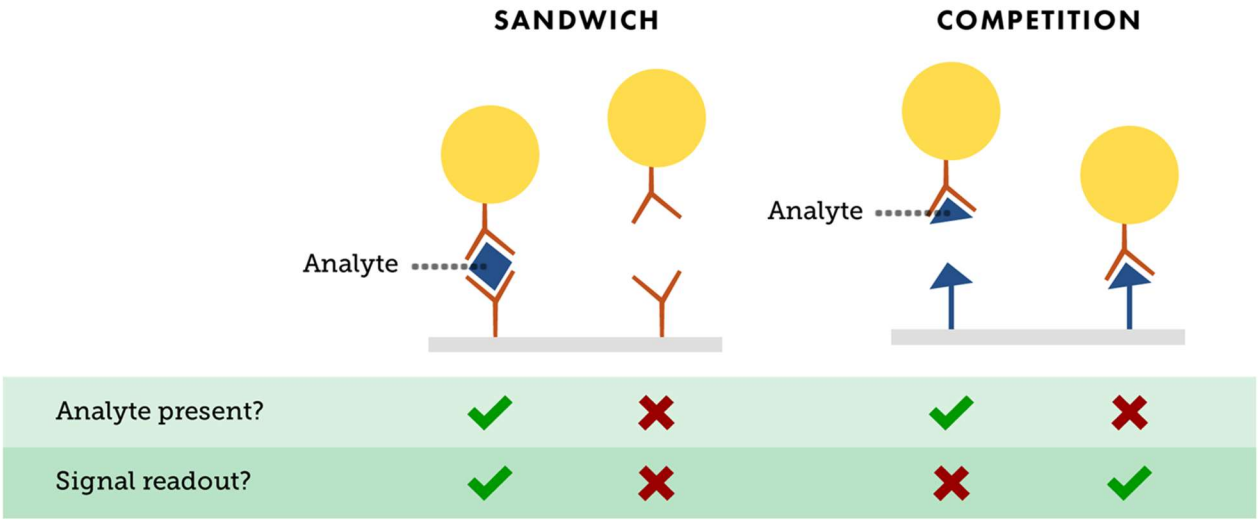

Figure 2: Difference between sandwich and competitive formats of LFDs [3].

Multiplexed LFDs

Both sandwich and competitive assays can be developed to include one or more test lines. In fact, the development of multiplex assays is easier in LFDs than for ELISA tests, mainly for technological reasons. However, the number of molecules detectable in LFDs is limited, while ELISA offers more possibilities (but these tests do not yet exist commercially).

A multiplexed assay may be used for:

- Detecting multiple targets in a single test rather than using many individual tests. In situations where only a small sample volume is available a multiplex assay allows you to maximize its use,
- To assist diagnosis where the presence of a number of markers together is required (example of aflatoxins),
- Confirming the presence of multiple contaminants during high volume food and feed testing,
- Offering cost-saving benefits to end-users in a laboratory or in-the-field by testing for different targets simultaneously, and
- Remote or agricultural areas where resources are limited and where multiplexed testing will save time.

In addition, by using complementary reader technology, sandwich and competitive multiplexed assays can produce quantitative results.

Quantitative rapid LFDs

Early versions of LFDs were predominantly qualitative assays. However, improvements in reagents, component materials, and reader technologies along with manufacturing processes mean quantitative results are achievable. In addition, the developments in reader technology and advancements in raw materials, such as labels, means a lateral flow rapid test can match the sensitivity of an ELISA assay.

References

1. Asensio et al. (2008). Determination of food authenticity by enzyme-linked immunosorbent assay (ELISA). Food Control, 19(1): 1-8

2. Maragos & Busman (2010). Rapid and advanced tools for mycotoxin analysis: a review. Food Additives & Contaminants: Part A, 27(5): 688-700

3. Available at: https://nanocomposix.com/pages/introduction-to-lateral-flow-rapid-test-diagnostics