SECTION 1
INTRODUCTION TO LATERAL FLOW DEVICES

Welcome to the first lesson of module 3. This lesson will focus on Lateral flow devices underlying principles and their advantages and disadvantages compared to ELISA tests.

LATERAL FLOW DEVICE

The Lateral Flow Devices (LFD) are common **Point of Care** (POC) diagnostic tools. It has been used in medicine, food sciences, agriculture, etc.

Like ELISA tests, LFD use the interaction between antibodies and an antigen (antibody-antigen binding) to detect the presence of the antigen in a liquid sample.

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Lateral flow devices are a paper-based technology invented in the late 80s and used for the detection and quantification of analytes in complex mixtures.
The term LFD regroups several denomination like Lateral flow test, Lateral flow device, Lateral flow assay, Lateral flow immunoassay, Dipstick but the underlying technology is always the same. They are Point-of-care diagnostic tools, which are tests that do not involve the use of laboratory staff and facilities to provide the result. LFD are currently used for qualitative, semiquantitative and to some extent quantitative monitoring in resource-poor or non-laboratory environments. For example, pregnancy home tests are LFD. Globally, a sample is placed on a test device and the results are available within 5–10 min.

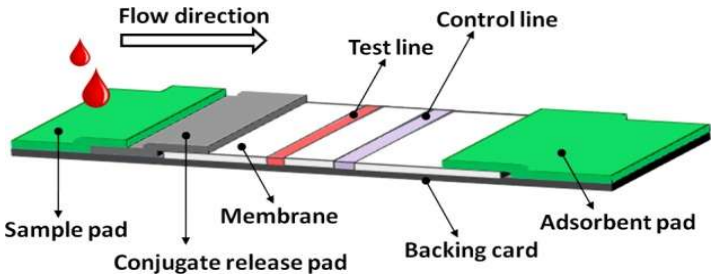
[Click twice for animations]

Like ELISA tests, LFDs use the interaction between antibodies and an antigen (antibody-antigen binding) to detect the presence of the antigen in a liquid sample. Consequently, they are considered as immunoassays. However, as the samples molecules moves along a membrane, LFD are in fact considered as immunochromatographic tests.

LATERAL FLOW DEVICE

Principle

Typical configuration of a LFD



From Koczula and Gallotta (2016)

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LFDs are usually composed of the following elements:

- a sample pad, acting as the first stage of the absorption process, and in some cases contains a filter, to ensure the accurate and controlled flow of the sample. To use the device, a liquid sample is added directly to the sample pad.

- a conjugate release pad, which stores the 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.

- a membrane. Nitrocellulose is by far the most commonly used material. As the sample moves along the membrane the binding reagents situated on the membrane will bind to the target at the test line and a coloured line will be produce. In quantitative assays, the density of the line will vary depending on the quantity of the target present. In that case, the LFD must be combined with a reader to provide quantitative results. The membrane is considered as the most critical element as it should have adequate and homogenous capillary forces but also the capacity of binding and immobilizing proteins to allow the detection of the targeted compound. It should be noted that LFD assays allow the detection of several analytes simultaneously. In that case, a test line is present for each targeted analyte. To the best of our knowledge, there are no

commercial multiplex LFDs for the detection of food contaminants.

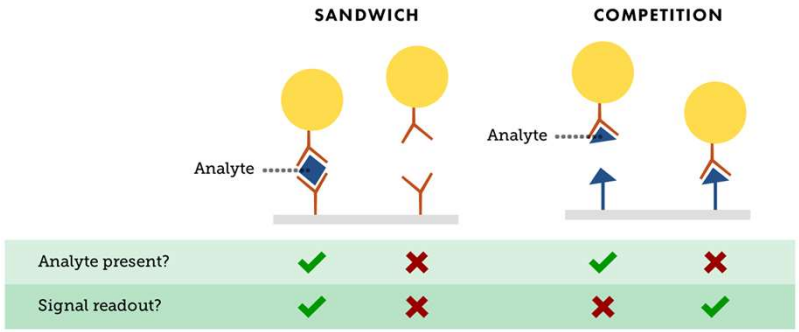
- Finally, the LFD contains an absorbent pad, used to collect the processed liquid. The absorbent pad allows the use of larger sample volumes, which results in increased test sensitivity. The most popular absorbent pads are made of cellulose filters.

These components (Sample pad, conjugate pad, membrane and absorbent pad) are usually fixed to an inert material.

LATERAL FLOW DEVICE

Principle

Two types of format



From nanocomposix.com

LFD are one-step assays, so once the sample is added to the sample pad, you just have to wait for the required time before the determination of the results, by visual inspection or by using a reader.

Like for ELISA tests, there are different types of LFDs. The assay formats can be either direct (sandwich) or competitive (inhibition).

Sandwich assays are typically used when testing for larger analytes with multiple antigenic sites. In this case, the test line is made up of immobilized antibodies that will interact with the antigen part of the complex antigen-labelled antibody. A positive result is indicated by the presence of a colored test line. In quantitative sandwich assay, the signal intensity of the test line is directly proportional to the amount of analyte present in the sample. To accurately measure the test line intensity, the LFD result must be analyzed by a strip reader.

On the other hand, competitive formats are typically used when testing for small molecules with only one epitope, which cannot bind to two antibodies simultaneously. In this case, the test line is made up of immobilized targeted analyte that cannot react with labelled antibodies if the targeted analyte is present in the sample. So, in this format, a positive result is indicated by the absence of a colored test line. In quantitative competitive assay, the signal intensity is *inversely* proportional to the amount of analyte present in the

sample.

In both format, a control line is needed to confirm that the assay is working properly. The control line contains immobilized targeted analytes, that will bind the conjugate with or without the wanted analyte present in solution.

LATERAL FLOW DEVICE

Labels

The most important requirements of the nanoparticle label include:

- colloidal stability in solution under various conditions and temperatures
- susceptibility for detection over a large dynamic range
- efficiency and reproducibility of conjugation
- lack of or very low non-specific binding characteristics (ensuring a high signal-to-noise ratio)

Labels use to produce the conjugate can be various in the case of LFDs, including gold, selenium or silver nanoparticles, colored latex beads, magnetic particles, carbon nanoparticles, organic fluorophores, textile dyes, enzymes and others.

Unlike ELISA tests, enzymatic detection is not the usual detection method as it would require additional steps to produce analytical signal. In the case of LFD, gold is actually one of the most used label as it produce a direct signal. Hence the labels which give direct signal are preferable in LFDs because of less time consumption and reduced procedure.

In any case, any material that is used as a label should be detectable at very low concentrations and should retain its properties upon conjugation. Ease in conjugation with biomolecules and stability over longer period of time are desirable features for a good label. These labels should also been commercial availability at low cost for commercial purpose.

LATERAL FLOW DEVICE

Advantages

- User friendly (no need for high training skills)
- No need for expensive equipment
- Cheap and stable
- Can be used on production site
- Sensitivity, specificity as good as the antibody allows it
- Can be used for quantitative results (a reader is required)

Compared to other tests, LFDs present several advantages:

The most obvious is that it is a user friendly format. LFD are one-step tests without washing steps. They can be performed on small volumes samples.

This kind of test does not need expensive equipment. They are stable at least a year and most of the time without refrigeration.

LFD are cheap and can be used on site compared to other diagnostic tools like ELISA or LC/GC-MS.

The results are obtained quickly, usually below 20 minutes, which means that these tests are useful to treat emergency.

Commercial LFDs are sensitive and specific enough to allow their use for regulatory purpose. Depending on the test, the results could also be quantitative or semi-quantitative.

LATERAL FLOW DEVICE

Disadvantages

- Reproducibility
- Potential limitations for several matrices (Viscosity, interferents, etc.)
- Restriction of total volume test induces a limit of sensitivity
- Limited POC interest for non liquid samples

Unfortunately, LFDs also presents flaws and limitations compared to others analytical methods.

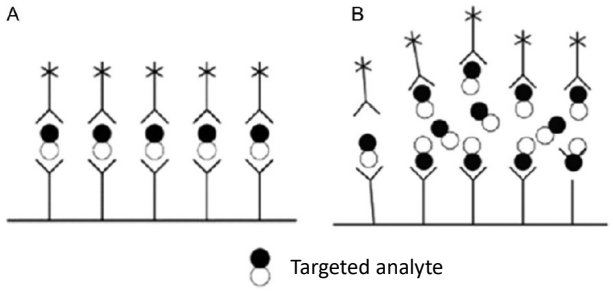
Several are due to the lack of sample preparation.
For example, the reproducibility of LFD results can be problematic, especially in non homogenous matrices or in matrices containing interferents. The presence of interferents can also introduce potential issue like the obstruction of the membrane pores. The viscosity of some food samples could also introduce capillary issues. Acid or basic food samples should also be neutralized to avoid further issues. In the same way, the limitation of the volume that can be added on the sample pad induces a limit of sensitivity. A sample preparation would be needed to reduce the volume of the food sample to increase the analyte concentration on the analysis sample. All these problems are the consequences of the testing methodology.

In the same way, LFDs have limited interest for POC applications in the case of non liquid samples. An additional step of sample preparation should be performed, which involved potential use of non portable equipment, like centrifuges or grinders.

LATERAL FLOW DEVICE

The **hook** effect

Adapted from Winder et al. (2017)



A major problem in LFD analysis are false negative results due to high analyte concentrations – known as overload effect or hook effect.

The A part of the schematic proposes what should happen in a classical LFD sandwich assay.
In the B part of the figure, high amounts of an analyte lead to an imbalance between the analyte and the antibody used, thus preventing the formation of the necessary sandwich complexes. Consequently, the formation of the test band is prevented and false negative results could be inferred.

To solve this problem, rapid test strips have been developed with an additional band, the so-called hook line. The hook line makes the overload effect visible. The attenuation or absence of the hook line indicates a very high content of allergen in the sample. This allows high concentrations of the analyte to be detected and false negative results to be identified.

SECTION 1
THE END

This covers the first part of the LFD module

SECTION 2
APPLICATION IN THE DETERMINATION OF DIFFERENT FOOD CONTAMINANTS

Welcome to the second lesson of module 3. In this lesson, we will discuss the place of LFD for the purpose of food contaminants detection and their place for food monitoring.

RELIABILITY OF TESTING

Fit for purpose approach

- Check if test is fit for purpose
- Fit for the food matrix?

- Potential cross reactivity with one of the ingredients?
- Potential interferents?

LFD are immunoassays, like ELISA tests, consequently, they have to follow a similar process in order to be used without issues

First, a lot of LFD are qualitative or semi quantitative which can be irrelevant if your objective is actually to obtain a contaminant concentration. Like it was said in the previous lesson, quantitative LFDs must be used with a reader to obtain a concentration value.

Next, as LFDs rely on antigen-antibody liaison, you need to check if ingredients in the food matrices will not induce cross reactivities. Usually, LFD are used at the beginning of the food production chain, and few ingredients are involved at this point, which make this validation quick. In fact, most of potential interferent have been analysed or checked by manufacturers before the commercialization of the LFDs, but, like for ELISA, it is still possible to find new cross reactivities with new or exotic ingredients, or for analysis for which the use of LFDs was not anticipated.

PLACE OF LFD IN FOOD ANALYSIS

To assess the risk

- Screening method (cheap, quick and high throughput) for mycotoxins, food allergens, phycotoxins, pesticides, veterinary drugs, etc..

To check compliance with or efficiency of food regulations

- Screening method to detect non compliant samples. A confirmatory method will be needed.

For quality control in food industries

Like ELISA tests, LFDs are used to assess the risk, as a screening method, for several food contaminants but mostly for mycotoxins, food allergens and pesticides. To the best of my knowledge, LFDs are not widely used for the detection of phycotoxins but LFDs have been developed for this purpose.

ELISA is also widely used to verify compliance with food regulations. Food industries are majors users as ELISA is affordable compared to chromatographic technologies. On the other side, regulatory agencies have to used reference methods and LFDs are not among the reference methods. However, like for ELISA methods, LFDs could be endorsed at a national level through national guidelines or compendium. LFDs can also be used as screening methods or for qualitative measurement when the absence of the targeted food contaminant is mandatory.

They are widely used in food industry for the validation of cleaning procedures. They can also be used in production sites where cross contamination can occur du to food processing or food preparation, like restaurants for example.

PLACE OF LFD IN FOOD TESTING

Major advantages

Point Of Care (POC) testing

- Easy to use
- Cheap
- No equipment needed
- No high skilled analysts
- Stable

Ideal for surfaces

- Validation of sanitation or cleaning in place
- Quality control in production site (industries, restaurants, etc..)

Now, if we go back to the interest of LFDs, in recent years there has been an increasing demand for point-of-care multiple diagnostic assays with multiple test lines allowing the rapid and simultaneous detection of multiple analytes present in samples. Such assays should be easy to perform without the use of laboratory equipment, or individuals trained in chemical analysis.

LFDs are very good candidates as they are cheap to produce, easy to use and, importantly, widely accepted by users and regulatory authorities. Moreover, because of the long shelf life and the fact that refrigeration is not required for their storage, LFDs are very well adapted for use in developing countries and remote regions. They can also be useful in atypical situations like battlefields.

LFDs are also ideal for the testing of surfaces which is almost impossible with others analytical methods without using swabs. Surfaces are not analysed on a regular basis by regulatory agencies but this feature is useful for quality purpose in industry.

LATERAL FLOW DEVICE

How to use them?

Without a reader

Before the use of the LFD, samples have to be put in a form that is convenient for the test, aka a liquid sample. Be careful, that the sample preparation should be done in accordance with the booklet instructions provided but the manufacturers. Usually, sample preparation is easy and quick to maintain the advantages of LFDs compared to other analysis techniques. However, solvents (like methanol or ethanol) could be required for the optimal extraction of a particular contaminant.

To perform an analysis, the test strip is dipped into a liquid sample. If the analyte is present in the sample, a colored line becomes visible on the test strip after a predetermined amount of time. In alternative LFDS formats, a few millilitres of the liquid sample must be deposed on sample well and the LFD stays at horizontal for the prerequisite amount of time.

This visual evaluation allows for qualitative and semi-quantitative analysis.

LATERAL FLOW DEVICE

How to use them?

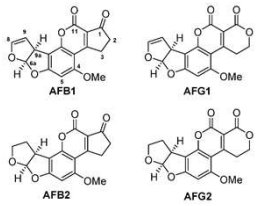
With a reader

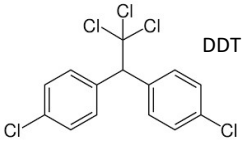
For qualitative measures, an LFD reader will be mandatory. Some producers have dedicated equipment (like the Vicam reader), others have developed alternatives. For example, some smartphone are now regularly used as reader by using their camera. In this case, the results are display on the screen of the phone.

MAIN APPLICATIONS

Major advantages

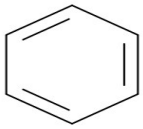
- Allergens
- Mycotoxins
- Phycotoxins
- Pesticides
- Veterinary drugs





DDT

Benzene



But also for bacteria and microbial toxins

Like ELISA assays, there are mostly used for allergens or mycotoxins/phycotoxins detections but their low development costs and ease of production have resulted in the expansion of applications, like pesticides, veterinary drugs or microbial toxins detection, across multiple test-sites where rapid tests are required. LFDs are now widely used as routine tools at the POC as part of an early-stage detection protocol, but positive results always need to be confirmed by analytical methods like LC or GC-MS.

Like said before they are useful for the testing of surfaces, which makes them essential in allergen management. It is also possible to use them for rapid detection of pesticides, as pesticides are spread in fields and are susceptible to be found on surfaces.

In fact, LFDs can be used for almost all food contaminants and it is very likely that new tests will be developed in the future years for new applications, including the detection of dioxins and furans. LFDs are still a relative emergent technology and recent development like the use of smartphones as reader will help to continue this development. However, the counter-effect is that several limitations still need to be overcome, like reproducibility issues, and new issue. will emerge as new applications will be imagined.

This covers the module about LFD. Do not forget to complete the self evaluation document before the start of module 4.