Online Training Curriculum

Confirmation Methods for Food contaminants

Principles of ELISA

Application in the determination of different food contaminants

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Overview

Utilisation of ELISA for the detection of food contaminants

In the last years, advances in ELISA technology have led to rapid development of different commercial immunoassays kits for applications in the food and feed industry. The simplicity of this type of tests and the short time required for the analysis make them suitable for food screening tests at a large scale.

Food allergens

ELISA plays a major role in food industry. It is the main platform for identifying food allergens such as those present in milk, peanuts, walnuts, almonds, and eggs, among others. Several other methods may be used but currently, the ELISA technique is the only one used in routine food analysis due to its high precision, simple handling and good potential for standardization. So far, mass spectrometry methods are still inaccurate for the quantification of allergens in food samples and are not used on a regular basis by small or medium business. An alternative to the ELISA format has gained importance in recent years - lateral flow devices - as they are very inexpensive, rapid, and portable, do not require instrumentation and are extremely simple to perform. However, the ELISA technique is still the most commonly method used in laboratories of the food industry and official food control agencies to detect and quantify hidden allergens in food.

Mycotoxins and phycotoxins

Most of regulations propose MLs for a limited number of mycotoxins (6 different mycotoxins for the *Codex Alimentarius*) and phycotoxins (none from the *Codex Alimentarius*, but 5 from the European Union). In this context, ELISA methods are useful tools for early detection and screening. When the number of targeted analytes is high, like for pesticides, dioxins and furans, operators have no choice but to use other techniques like UPLC or GC coupled with mass spectrometry.

Commercially available ELISA kits for the detection of mycotoxins and phycotoxins are normally based on a competitive assay format that uses either a primary antibody specific for the target molecule or a conjugate of an enzyme and the required target. These kits can be portable, rapid and are highly specific as well as simple to use. However, the disadvantage of these kits, compared to other potential methods like HPLC, lies in the fact that they are for single use, which can increase costs of bulk screening. Additionally, competitive ELISA suffers from having a limited detection range due to the narrow sensitivity of the antibodies, be they mono- or polyclonal. The development of antibodies for most mycotoxins, due to their small size, require development of a carrier molecule usually, a protein (e.g. bovine serum albumin), to achieve immunogenicity. The conjugation process can also be responsible for decreases in assay selectivity.

When positive results are observed, a second analytical method (UPLC or GC-MS mostly) is used to confirm or invalid the results. However, it must be considered that in case of conflicting results, there is a need for a valid reference method.

Food adulteration

Food fraud may occur when food is misrepresented. It can pose serious health risks if, for example, unidentified allergens or hazardous materials are added to food products. It can also have an economic impact on the buyer (for example, paying for a product that is actually of lower quality). In some cases, food contaminants are added to the product via adulterants and can therefore serve as a marker of adulteration. Because of that, screening methods like ELISA have been proven useful for the detection of adulterants like forbidden food dyes [1] or melamine [2].

Key parameters

Specificity and sensitivity

The specificity of an analytical assay helps determine the likelihood of non-specific binding in an assay. This also makes it possible to validate the effectiveness of the blocking procedure in the applied protocol. Determining the sensitivity and specificity of a method can be done in two different ways. The first is to use standard reference materials, to see if the test can be used in the food industry in a reliable way. This verification is always carried out by the test producer, before the commercialization of the kit. The second is the evaluation of the test with real food samples, which is very important for routine testing. True positive and negative replicates of real food sample should be used to control these key parameters. Negative samples are not the only control samples that should be used in an assay, especially for routine testing. Especially because sometimes the assay results present negative readout, while the target analyte is in fact present in the samples. Therefore, obtained results can be classified into the following categories:

- True positive: the sample is considered positive and actually contains the target analyte,
- True negative: the sample is considered negative and does not contain the target analyte,
- False positive: the sample is considered positive but does not contain the target analyte
- False negative: the sample is considered negative but contains the target analyte.

Accuracy

Another important key parameter, in order to correctly evaluate the results of a test, is accuracy. Accuracy is usually calculated by evaluating the produced results against the total number of repetitions performed. In addition, these tests should be conducted using predetermined concentrations of the target analyte, which allows the production of calibration or standard curves. The squared correlation coefficient (R2) of these graphs provides a statistical model to study the precision of the test. R2 varying from 0 to 1, the closer the value of R2 is to 1, the more the test has

a high level of accuracy. The analysis of the standard curve is one of the essential steps to check whether a test is reliable or not.

Limit of detection and Limit of Quantification

Other important parameters are Limit of detection (LOD) and Limit of Quantification (LOQ). The LOD is the lower most quantity of a targeted analyte that the assay can possibly detect with assured confidence. The LOQ is the lowest concentration at which quantitative results can be reported with a high degree of confidence. Both are calculated from Limit of Blank (LOB). LOD is defined as three times the value of the blank sample, and LOQ is defined as 10 times the value of the blank sample. For a signal at the LOD, the probability of false positive is small but the probability of false negative is important. At the LOQ, the risk of false negative is weak (less than 5%).

Moreover, quantification with ELISA brings another key point which is the measurable units which depend on target analyte. Commercialized kits may provide the measurements based on the United State Food and Drug Administration, the United States Pharmacopoeia, the World Health Organization, or the European Pharmacopoeia. Those are adopted common standard measuring systems, which are normally presented as units/ml, sometimes referred to as International Units (IU/ml) or ELISA Units (EU/ml) [3].

Performing a reliable assay

Even before the use of commercial kits, several points have to be noted. First, the production of ELISA kits for food industry applications is challenging as a selection of adequate control and standard samples is necessary to carefully calibrate the assay. Secondly, ELISA tests are often validated by manufacturers for a limited number of food matrix, and further validation need to be done by the users, sometimes with the help of the manufacturer himself. Although firms manufacturing ELISA kits provide the assay validity parameters on their kit catalogs, it is sometimes difficult to obtain these data. Therefore, laboratories must test the assay validity parameters when they use a new ELISA kit. In case of new food matrix, it is very important to test individually each ingredient

individually to eventually report cross reaction of antibodies with those ingredients. Most of the time, manufacturers present the list of ingredients that can react and induce false positive results, but this list is always not exhaustive as new ingredients are develop on a regular basis. For example, mahaleb (a specie of prunes) induce false positive results with present in samples tested for almond detection [4].

Accordingly, for the purposes of standardization, it is important for a laboratory to use the kits of the same firm. Otherwise, you have to conduct the assay validity experiments for the new ELISA kit. Use different kits from different companies without any validation is among the most common analytical errors. This is mostly du to the fact that different companies will use sometimes different antibodies, different buffer or protocol extractions and sometimes even different ELISA type, leading to slightly different results. Some kits have been endorsed by the AOAC International, for specific food matrix and targeted analyte, and should be privileged compared to other kits.

Nowadays, many companies offer a wide range of ready-to-use ELISA kits that include antibodies, blocking buffer, substrate solutions and other necessary components. Such packages simplify the procedure and ensure a reliable assay just by closely following the instructions. Reagent management is an important aspect of conducting the assay. The solutions must be prepared before the analysis and without exceeding the volume necessary for the execution of the planned protocol, to avoid waste. If there is an excess of reagent solutions, they should be stored properly or discarded after use, if storage is not possible or recommended.

Nonetheless, errors are inevitable even in the well-established commercially available assays. In particular, there is a higher chance of mistakes when the protocol should be designed and conducted from scratch for economical reasons, or when a new protocol is being developed based on previous results. Most of common errors in ELISA can be avoided by having a clear knowledge of the sources of such errors. The suppliers and distributors often provide the necessary guidelines and troubleshooting strategies to resolve potential issues and to carry out successful assays.

References

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