

6. Methods of detection and quantification of Aflatoxins

- Determination of aflatoxins concentration in feeds and foodstuff is very important due to its toxicity and potency
- Since aflatoxins occur in low concentration in feeds and foodstuffs, analytical methods for the detection and quantification of aflatoxins have to be:-
 - ✓ specific,
 - ✓ sensitive, and
 - ✓ simple to carry out
- There are several methods used in the detection and quantification of aflatoxins in foods, including:-
 - ✓ thin-layer chromatography (TLC)
 - ✓ high-performance liquid chromatography (HPLC)
 - ✓ mass spectroscopy (MS)
 - ✓ enzyme-linked immune-sorbent assay (ELISA)

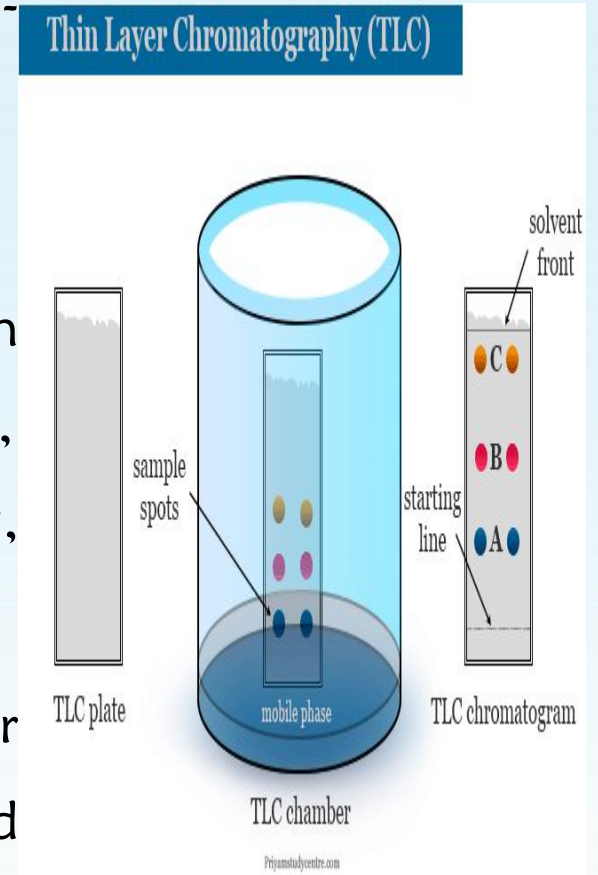


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- Each of these methods has advantages and weaknesses in aflatoxin analysis, mostly according to sensitivity, easiness of use, and cost-effectiveness

Thin-layer chromatography (TLC)

- TLC is one of the most widely used separation techniques in aflatoxin analysis, It comprises a stationary phase made of either alumina, silica, or cellulose immobilized on an inert material such as plastic or glass, called the matrix
- The mobile phase is comprised of acetonitrile, methanol, and water mixture, In TLC, the distribution of aflatoxins between the mobile and stationary phases is based mainly on differences in solubility of the analytes in the two phases



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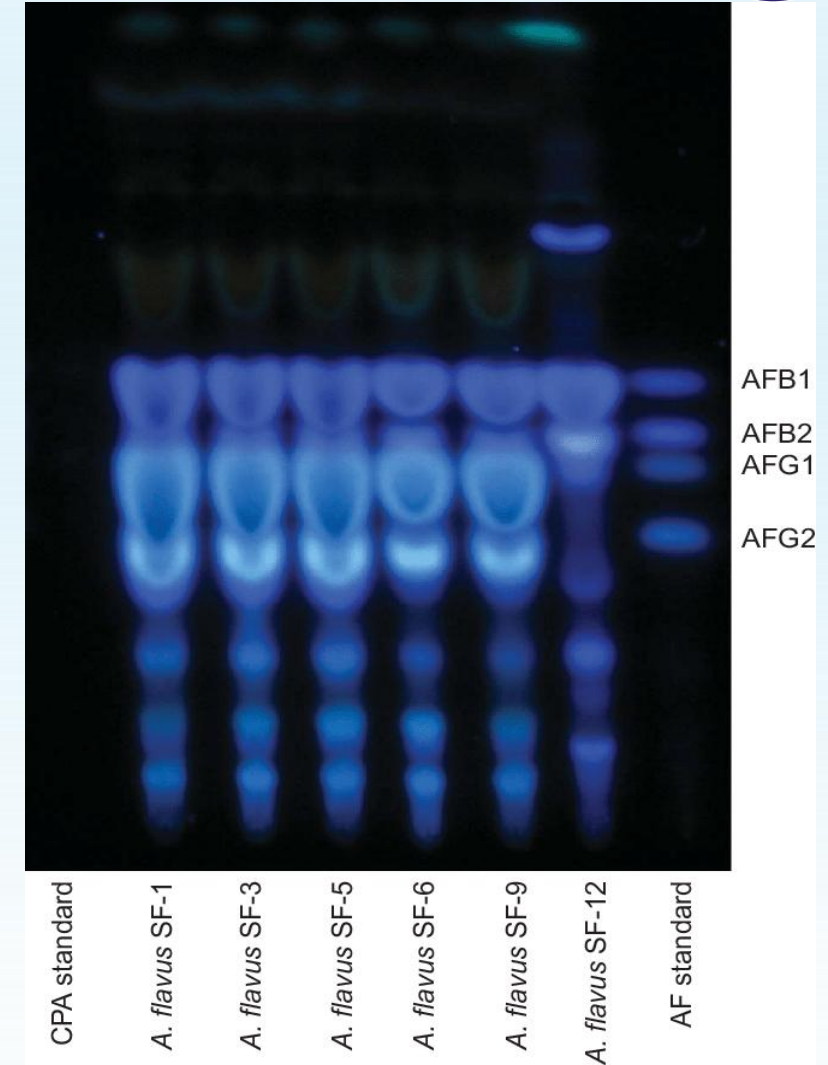
- Different analytes, depending on their molecular structures and interaction with the mobile and stationary phases, either adhere to the stationary phase more or remain in the mobile phase
- By this means allows for quick and effective separation

Advantages:

- The TLC technique can detect numerous types of mycotoxins in a single test sample
- TLC has excellent sensitivities

Weaknesses:

- It requires sample pretreatment and a skilled technician
- TLC lacks precision due to accumulated errors during sample application, plate development, and plate interpretation



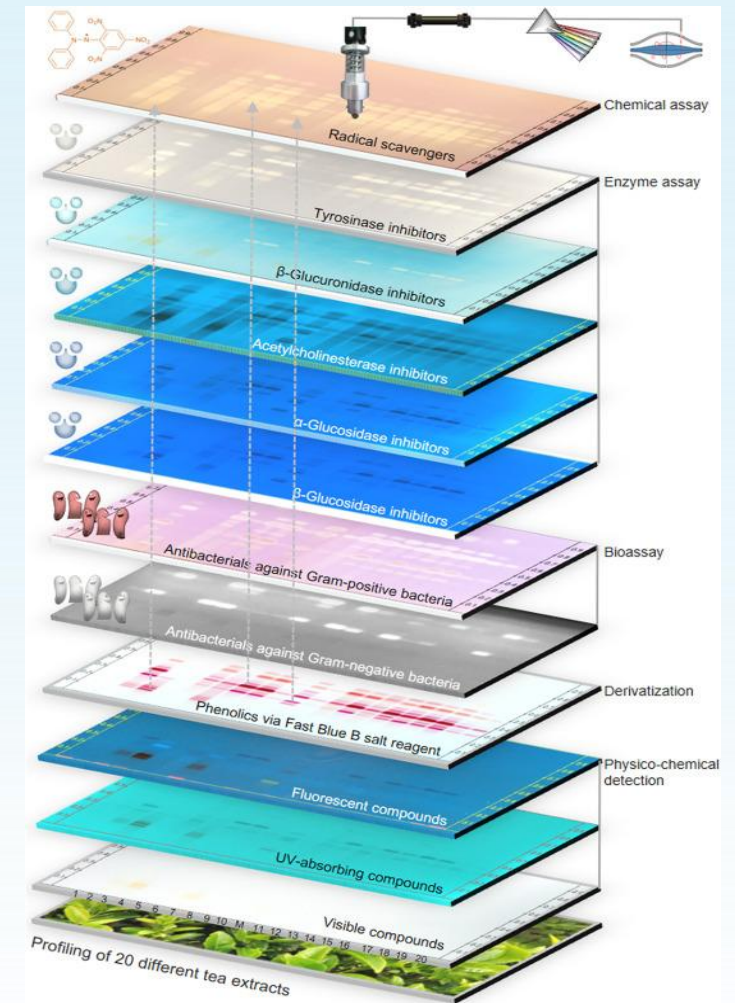
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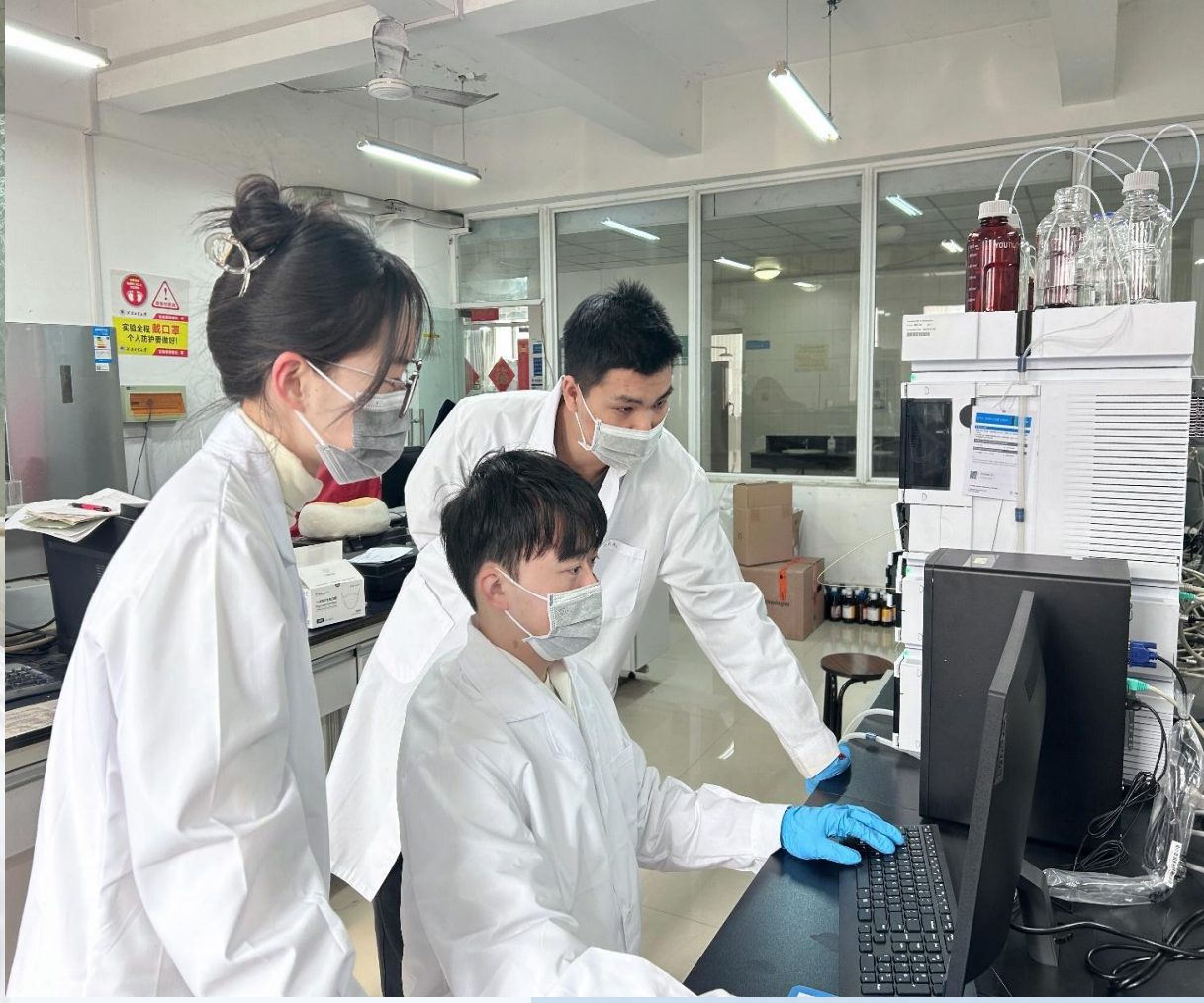
High-performance thin-layer chromatography (HPTLC)

- The improvement of TLC has led to high-performance thin-layer chromatography (HPTLC) which is the automated form of TLC
- HPTLC solved the problems associated with conventional TLC techniques through automation of sample application, development, and plate interpretation

Weaknesses:

- It is expensive and requires skilled operators
- It needs extensive sample pretreatment





HPLC in our Lab



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High-performance liquid chromatography (HPLC)

- HPLC is the most popular chromatographic technique for separation and determination of organic compounds. Also, is one of the most common methods used in detecting and quantifying aflatoxins in food
- The HPLC technique makes use of a stationary phase confined to either stainless steel, plastic, or glass tube and a mobile phase comprising aqueous/ organic solvent which flows through the solid adsorbent
- During analysis, the samples to be separated have different affinities for the two phases and thus move through the column at different rates. Programmable detectors i.e., FLD, VWD, or DAD may be used in the detection and identification of aflatoxins



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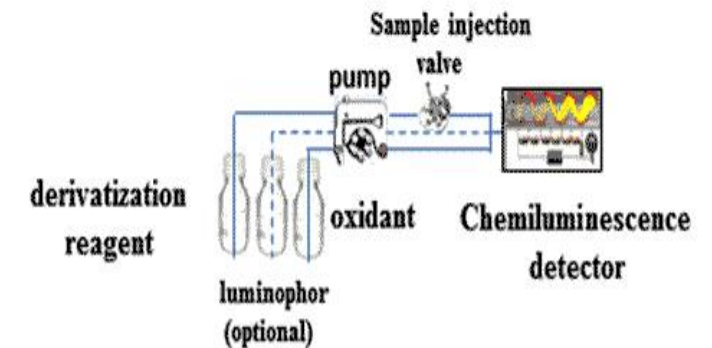
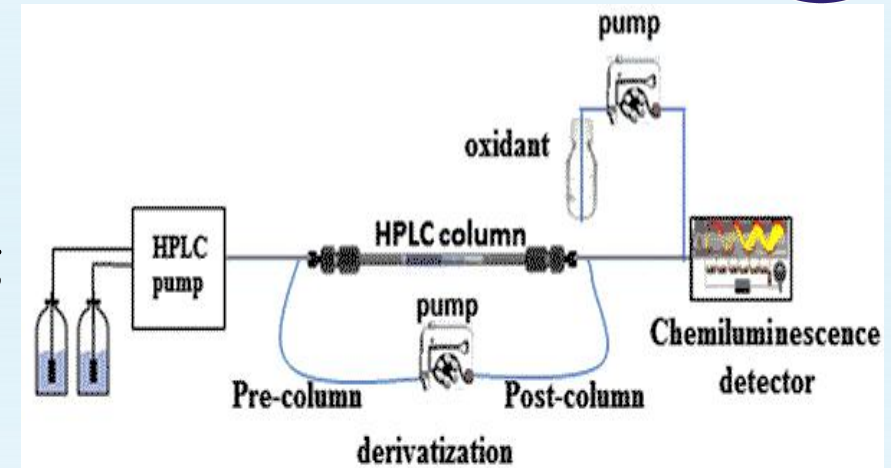
Advantages:

- HPLC provides fast and accurate aflatoxin detection results within a short time
- Has very low sensitivity of detection i.e., 0.1 ng/Kg using FLD

Weaknesses:

- HPLC for aflatoxins analysis requires rigorous sample purification using immune affinity columns
- HPLC requires tedious pre- and post-column derivatization processes to improve the detection limits of AFB1

To solve the issue of derivatization, nowadays the HPLC is coupled to the mass spectroscope i.e., HPLC-MS/MS



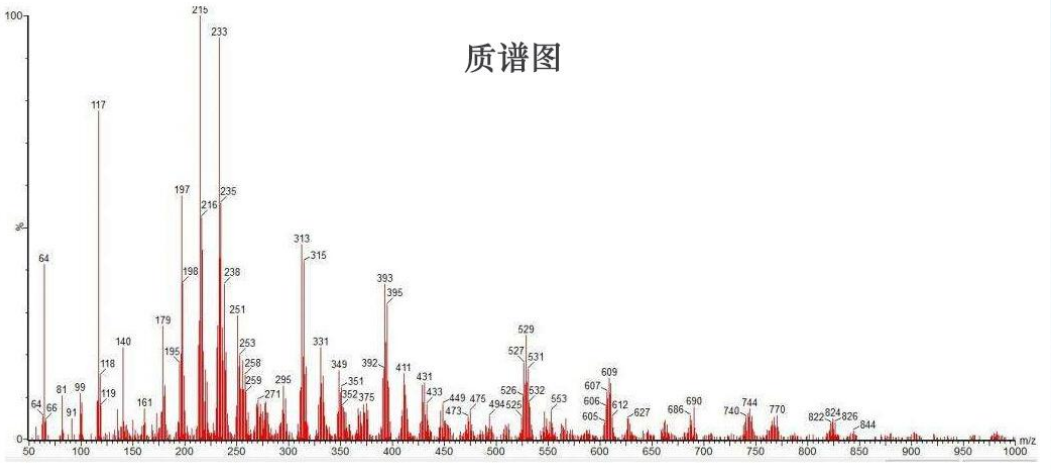
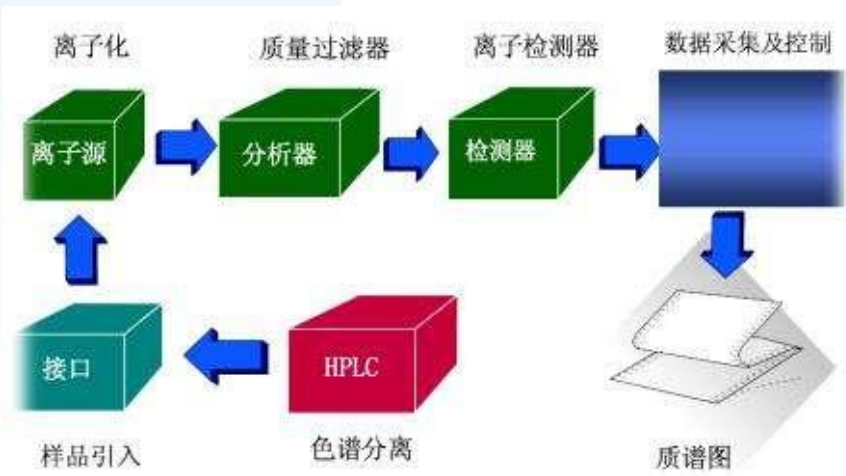
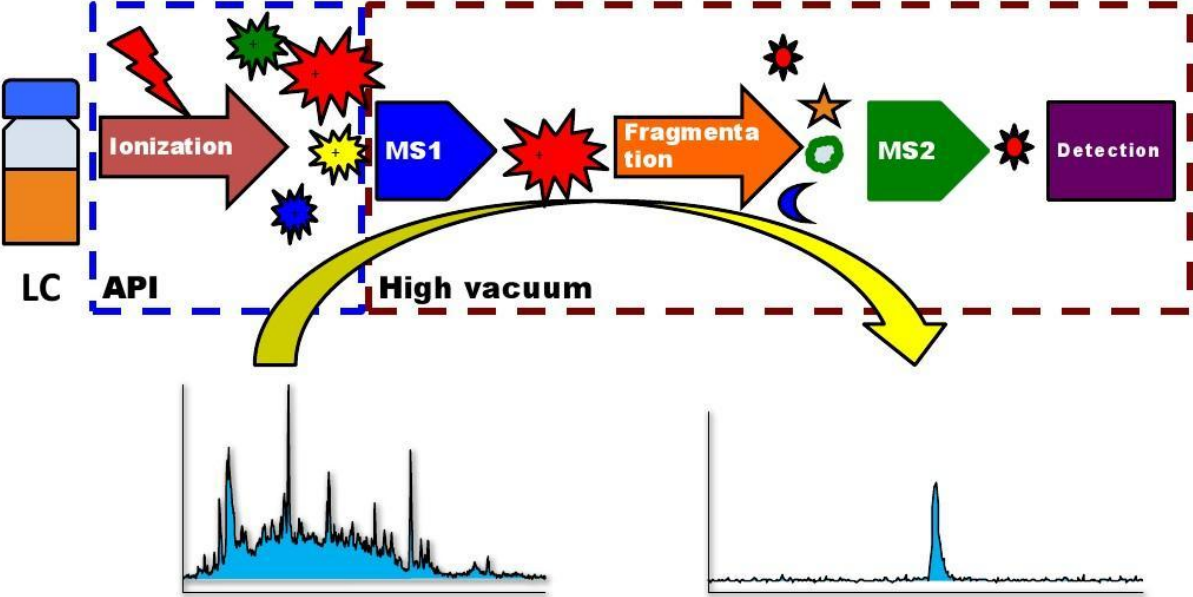
HPLC-MS/MS technology combines the high efficiency separation ability of chromatography and the high sensitivity of mass spectrometry in the instrument, which can simultaneously analyze multiple aflatoxins.



HPLC-MS/MS

LC-MS/MS工作原理

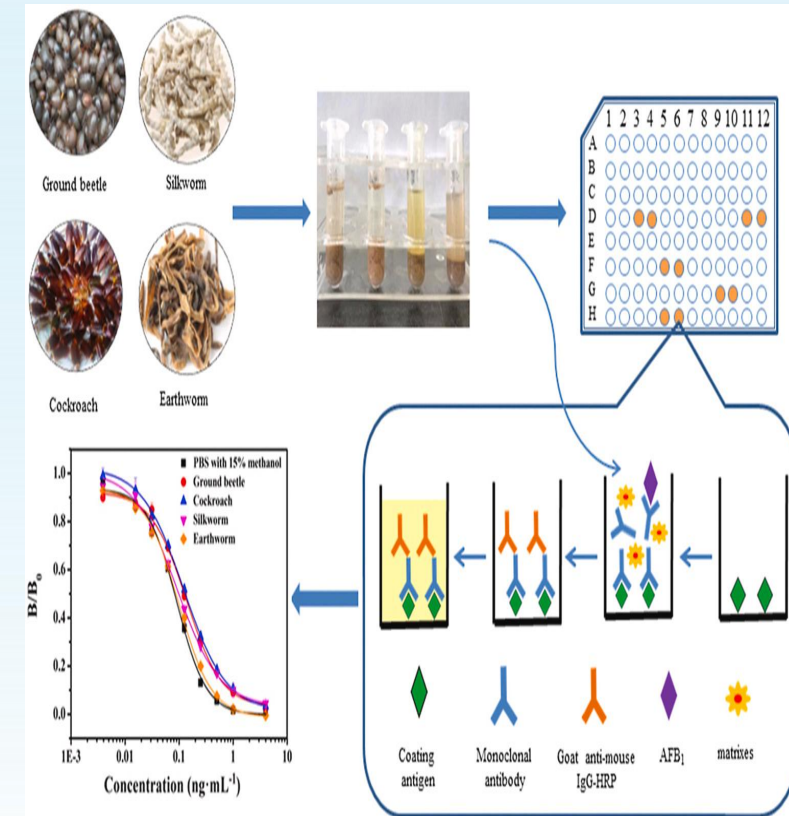
LC-MS/MS operating principle



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Enzyme-Linked Immunosorbent Assay (ELISA)

- ELISA is one of the major immunochemical methods used in aflatoxin analysis. The principle of enzyme immunoassays relies on the specificity of antibodies for antigens
- The sensitivity of the assay is increased by labeling either the antibodies or the antigens with an enzyme that can be easily assayed by the use of specific substrates
- Therefore, an antibody immobilized onto a solid support may capture an unlabeled antigen in the analyte, which is afterward detected by a labeled antibody
- Most of the kits use alkaline phosphatase (AP) and horseradish peroxidase (HRP) enzymes as labels in the analysis of aflatoxins



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Advantages:

- Allows testing of many samples (96-well assay platform) simultaneously
- ELISA kits are easy to use, cheap, and do not require extensive sample cleanup
- There are no inherent health hazards associated with enzyme labels as there are for isotopes

Weakness:

- The detection accuracy of ELISA technology is not high, and the detection results are affected by many factors, and there may be false positive or false negative results.



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Thank You

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