

Mass Spectrometry Vs. Immunoassay

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Guiding Question:

Can LC-MS completely replace Immunoassay in the clinical laboratory?

Objectives:

- ❖ To describe and define the lab techniques of Liquid Chromatography/Mass Spectroscopy & Immunoassay.
- ❖ To compare and contrast the uses of LC-MS and Immunoassay.
- ❖ To construct a pro/con table for each technique.

Immunoassays

Purpose: To Identify and quantitate specific antigens in a sample

❖ Chemical tests used to detect or quantify a specific substance, the analyte, in a blood or body fluid sample, using an immunological reaction.

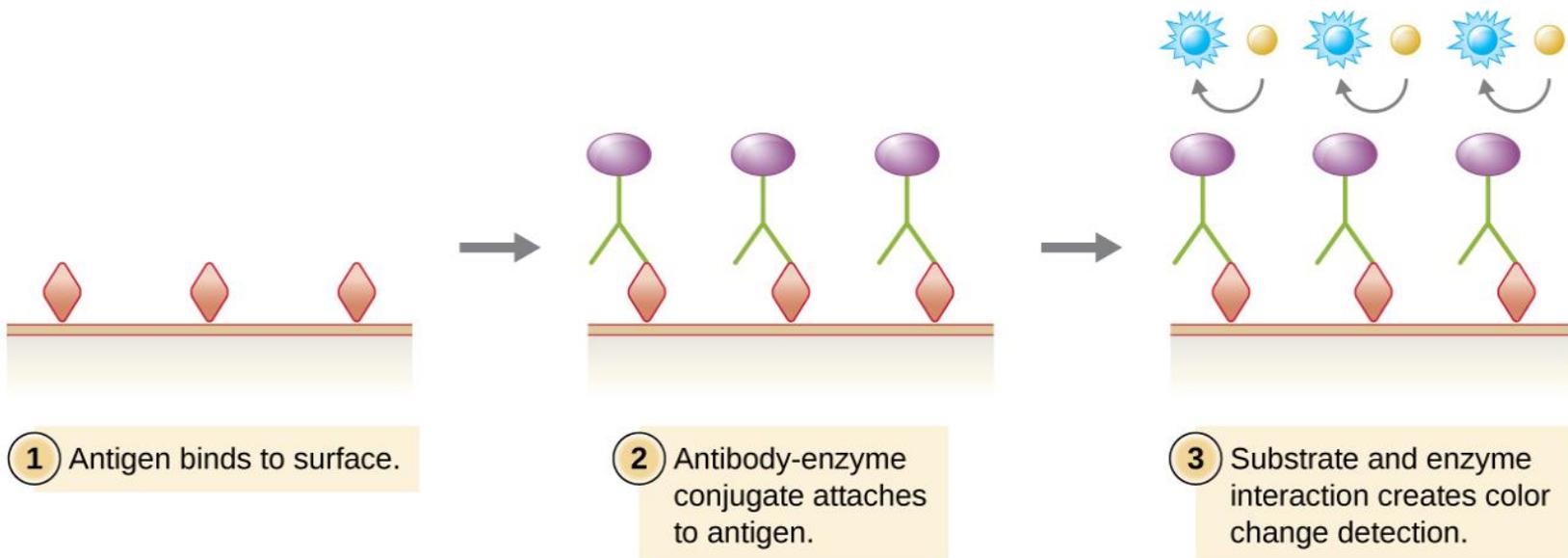
Uses: Cancer markers, diagnosing infectious diseases, cardiac analysis, therapeutic drug monitoring, and allergy testing.

[Immunoassay Explained](#)

Immunoassays

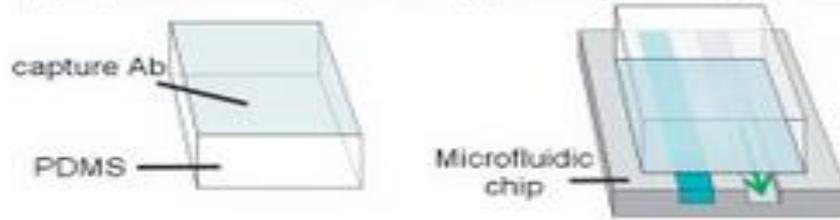
Technique: Highly sensitive and specific due to the use of antibodies and purified antigens as reagents.

- ❖ Automated and standardized.
- ❖ The amount of analyte concentration is obtained as compared to a reference standard curve.

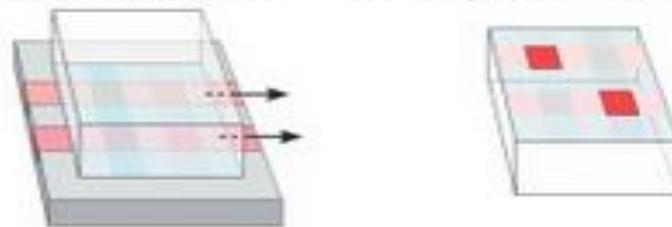


(A) Principle of the assay

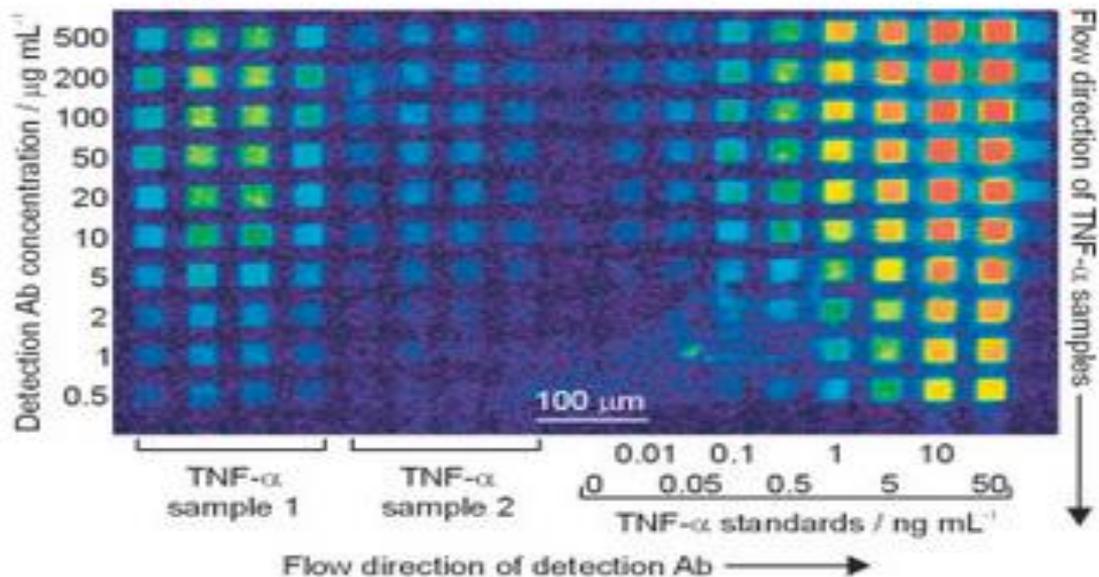
(1) deposition of capture Ab (2) delivery of analyte solutions



(3) delivery of detection Ab (4) imaging of the mosaic



(B) Detection of TNF- α with 1 pM sensitivity



Immunoassays



Advantages:

- ❖ Well characterized and trusted.
- ❖ Relatively inexpensive equipment, flexible and scalable(manual to automated).
- ❖ Training requirements low for basic daily operations.
- ❖ Good sensitivity and selectivity. (Multiple antigens)

Immunoassays

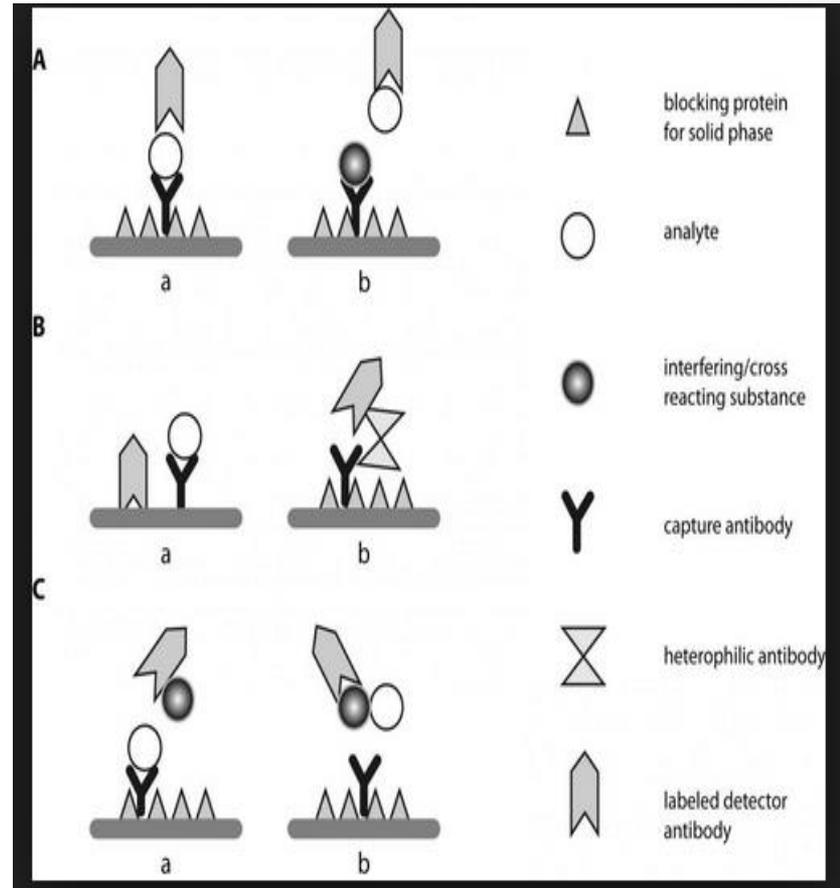
Disadvantages:

- ❖ Not all antibody/antigen pairs are readily available or exist or may have low sensitivity(Range limited). Ex:Testosterone
- ❖ Multi-step process with lot variations limiting inter and intra lab reliability.
- ❖ Higher cost for reagents and consumables.

Immunoassays

Disadvantages continued:

- ❖ Sample volumes can be quite high.
- ❖ Time consuming due to multiple step process(1-3 hours).
- ❖ Susceptible to interference.



Liquid Chromatography/ Mass Spectroscopy

Purpose: To Identify and quantitate specific molecules in a sample.

- ❖ High Performance Liquid chromatography: Separates out analytes in complex mixtures based on size and affinity for
- ❖ Mass spectrometry: analytes and chemical species in the sample are ionized and the ions separated based on their mass-to-charge ratio and subsequently detected.

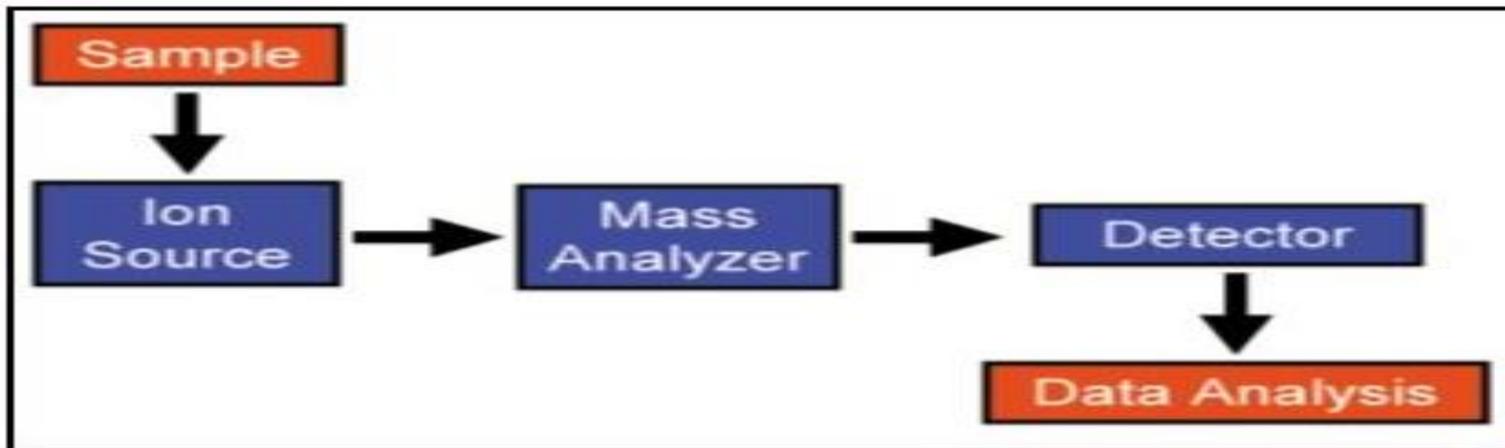
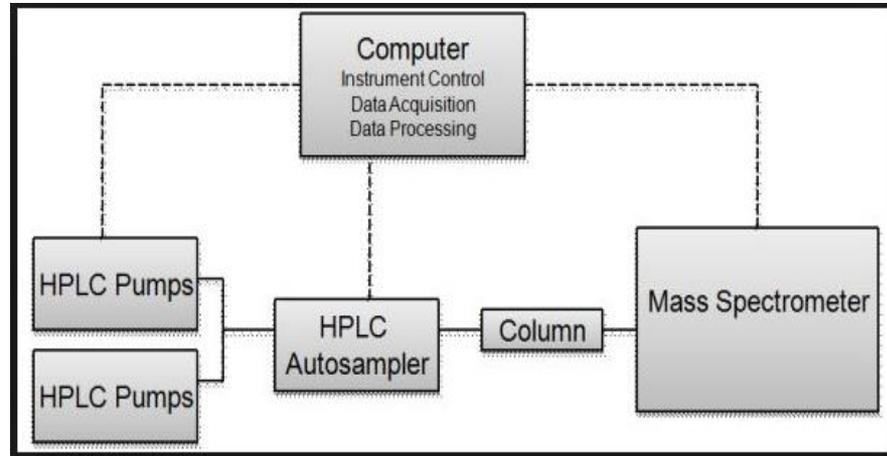
Uses: Newborn screening for metabolic diseases, therapeutic drug monitoring, drug of abuse and pain management testing, Endocrinology and steroid testing.

[Mass Spectrometry Explained](#)

Liquid Chromatography/ Mass Spectrometry

Technique: Measures mass to charge ratio of a molecule and its ionized fragments.

- ❖ Assays developed in house.
- ❖ Used for research as well as clinical assays.



Liquid Chromatography/ Mass Spectroscopy

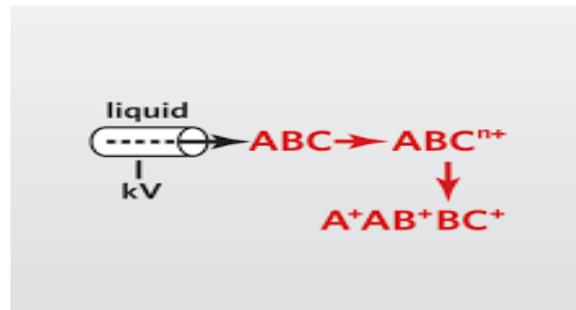
Advantages:

- ❖ Extremely high sensitivity and precision for identification and detection of analytes.
- ❖ Required sample volume extremely small (<5 μ l) (newborns).
- ❖ Day to day costs low. Chromatography column and solvents.
- ❖ Almost completely automated. High precision instruments ensure high Intra and inter assay reproducibility
- ❖ Wider range of compounds and analytes can be analyzed (proteins).
- ❖ Flexibility to create new assays.

Liquid Chromatography/ Mass Spectrometry

Advantages continued:

- ❖ Fast and produces a large number of quantitative and qualitative results. Many analytes can be analyzed at a time for a single LC-MS analysis of a sample.
- ❖ Not limited to biomolecules and can identify and quantify a wide range of organic and inorganic compounds.
- ❖ Selectivity in LC is good or easy to optimize compared with immunoassay.



Liquid Chromatography/ Mass Spectroscopy

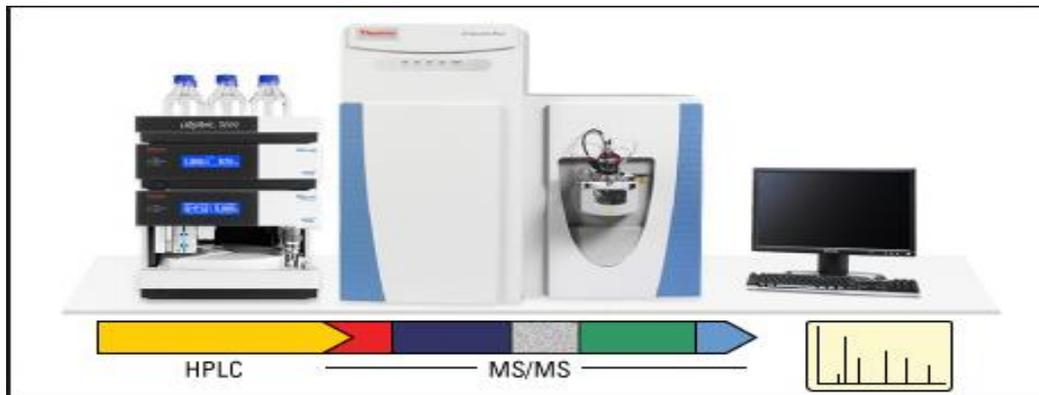
Disadvantages:

- ❖ New assay development validation process time consuming and complex.(Paperwork!)
- ❖ Lack of standardization when using “home grown” assays.
- ❖ Requires skilled staff and heavy training commitment.

Liquid Chromatography/ Mass Spectrometry

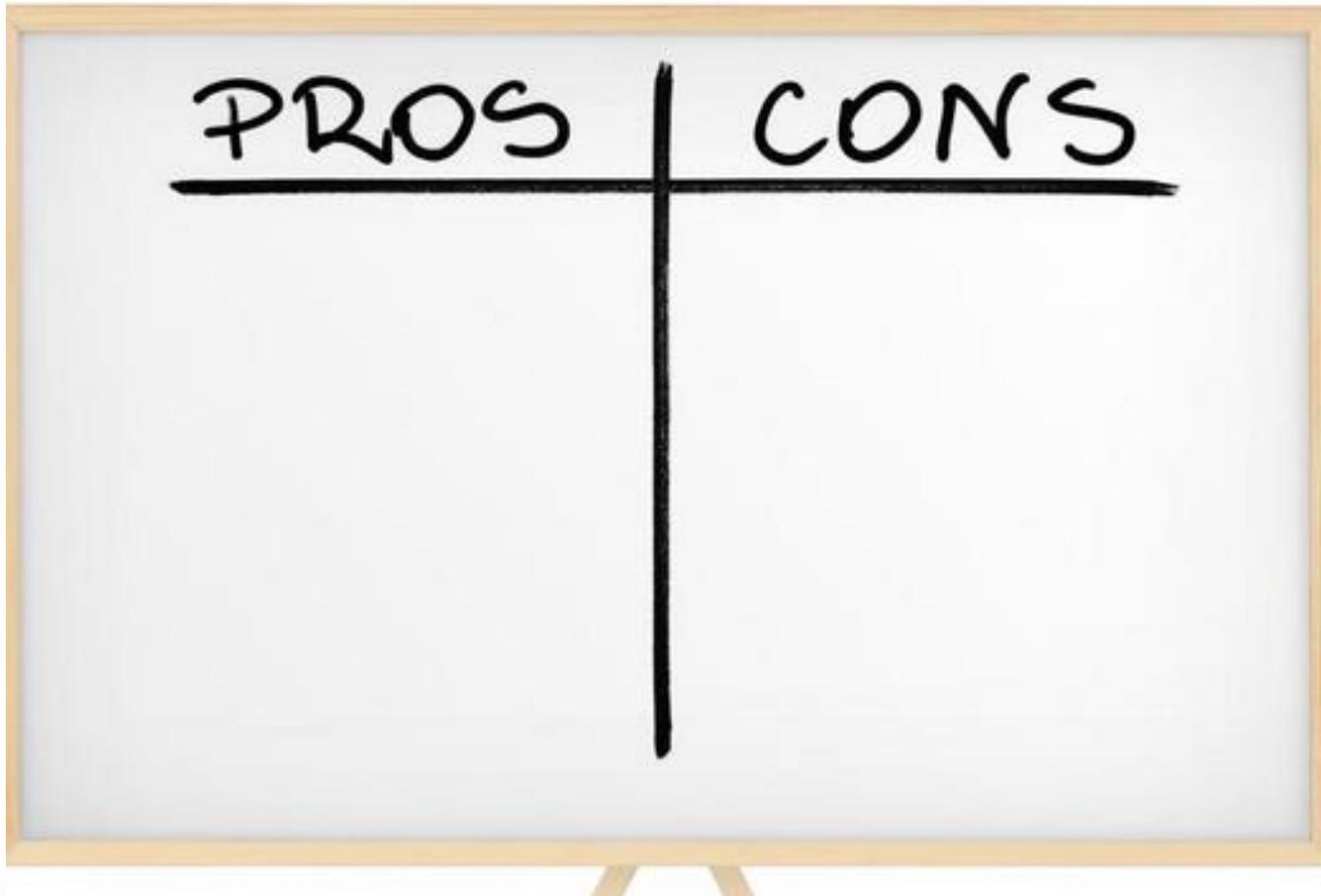
Disadvantages continued:

- ❖ High cost of initial investment and maintenance of instrumentation.
- ❖ Can be difficult to incorporate into core laboratory automation and bidirectional LIS for reporting.
- ❖ Sample Complexity-plasma has multiple proteins.



Compare & Contrast

Create a pro and con list for each technique



Compare & Contrast

Immunoassay

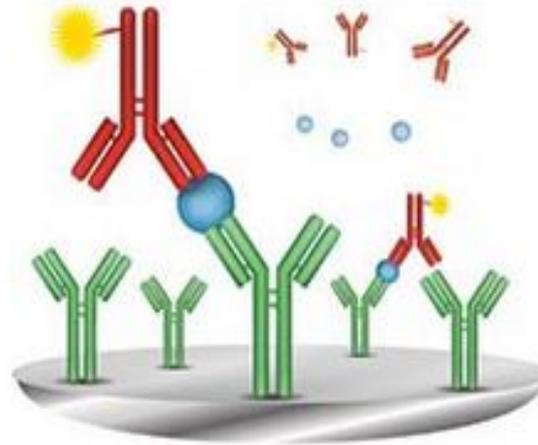
One antibody captures the analyte and a second labeled antibody binds to the analyte, forming a sandwich. Many types of signaling mechanisms can be used.

Pro: Cheap and easy

Con: Antibodies can cross-react with other analytes

Con: Other antibodies can interfere with the test

Con: Difficult to be sure what is being detected



Yellow = signal
Red = detection antibody
Green = capture antibody
Blue = analyte

SHUTTERSTOCK

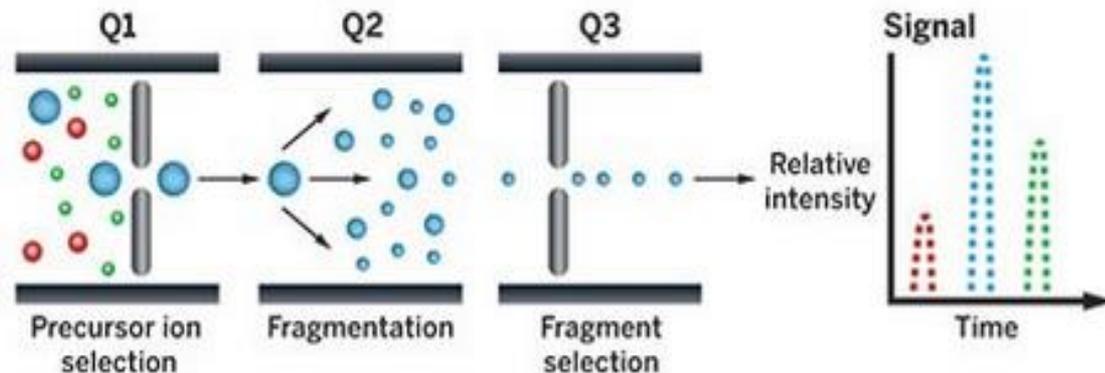
Mass spec

In the clinic, the most common analyzer on a mass spec, used to detect molecular fragments, is the triple quadrupole. The first quadrupole (Q1) in the analyzer selects a precursor ion, which then gets fragmented in the collision cell. Specific fragments are selected in the third quadrupole (Q2) for detection.

Con: Expensive instruments

Pro: Fragmentation pattern provides structural information

Pro: Better precision and accuracy than immunoassays



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Final thoughts...

- ❖ Can LC-MS completely replace Immunoassay in the clinical laboratory?

- ❖ Can the two techniques be combined????