

Gluten Testing in Food

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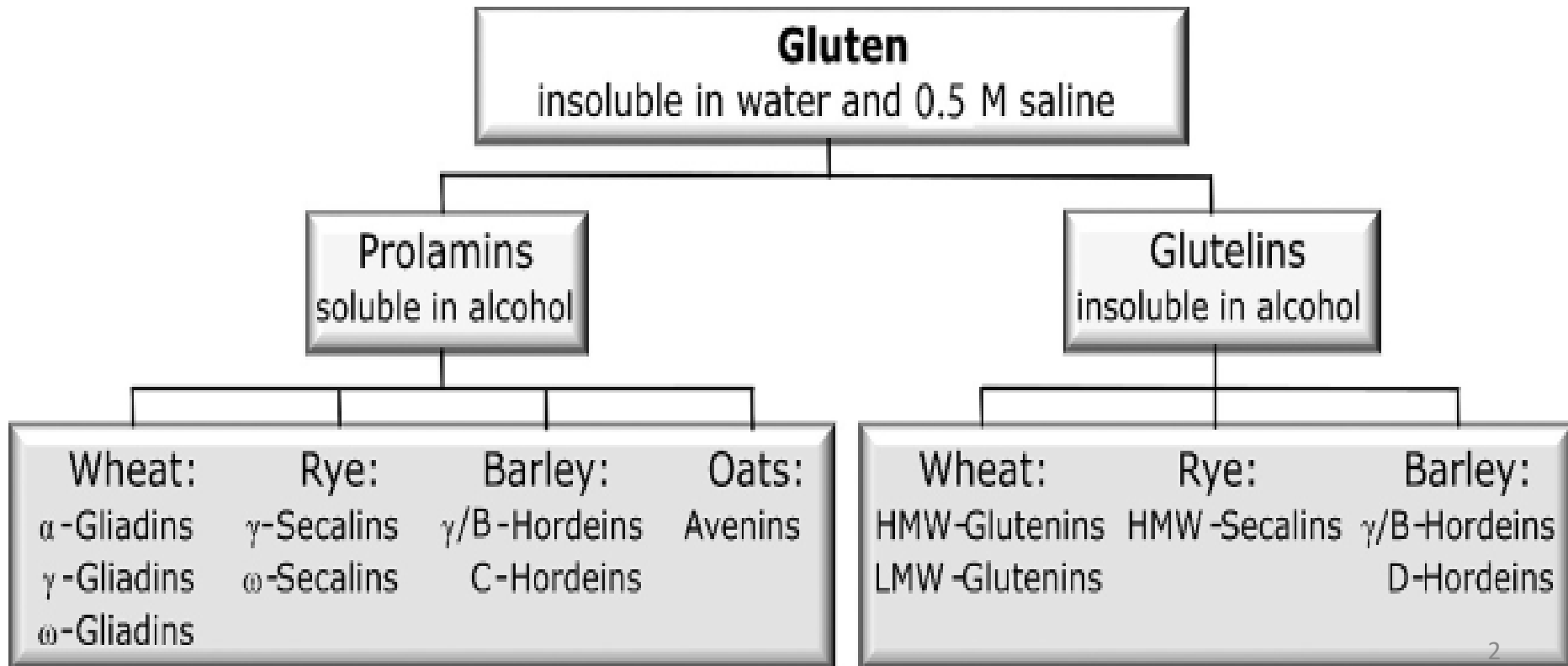
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Gluten

Chemically, Gluten proteins in **wheat** include ; gliadins as well as high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight (LMW) GS.





Gluten Intolerance

VS



Celiac Disease

- Not autoimmune
- No damage to small intestine
- No identified biological markers
- Symptoms triggered by consuming gluten
- Treated by a gluten free diet

- Autoimmune
- Causes damage to small intestine
- Genetic
- Symptoms triggered by consuming gluten
- Treated by a gluten free diet



Celiac disease

Celiac disease, wheat sensitivity, and allergy represent, which may occur in genetically predisposed individuals on the ingestion of wheat and derived products

According to the Codex definition, any food product containing **>20 mg/kg** gluten cannot be considered or labeled as “**gluten-free**” .

The only known treatment so far is a life-long gluten-free diet, which is almost impossible to follow because of the contamination of allegedly “gluten-free” products.

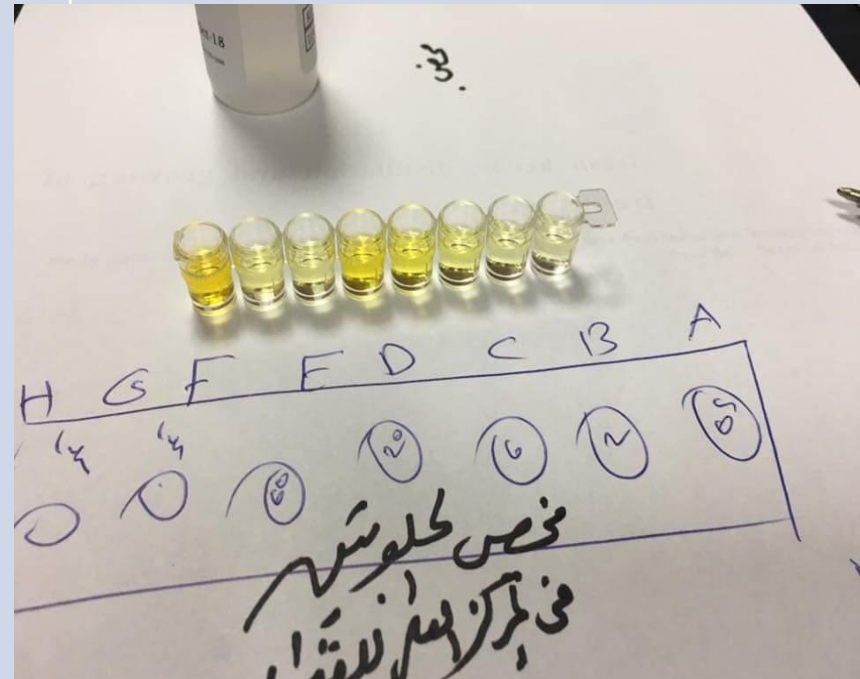


Testing for Gluten

1. Over the years, several gluten-detection and quantification methods have been developed and tested using the gluten-containing
2. Detect gluten contamination in different raw and processed foods will guarantee the safety of the foods for celiac patients.
3. There are an advantages and disadvantages of different gluten detection methods

These procedures can be classified into;

- Genomic
- Proteomic
- Immunochemical methods



Among genomic methods
(PCR) Polymerase Chain Reaction
based assay relies on the determination of specific DNA sequences.

Developments of testing

- The PCR-based assay was first applied by (Allmann et al. 1993)
- (Dahinden et al. 2001) developed a quantitative competitive (QC-) PCR system.
- (Henterich et al. 2003) developed a real-time immuno-PCR assay for gliadin detection
- Mujico and collaborators [2011] developed a highly sensitive RT-PCR based system for gluten detection in raw and processed samples.

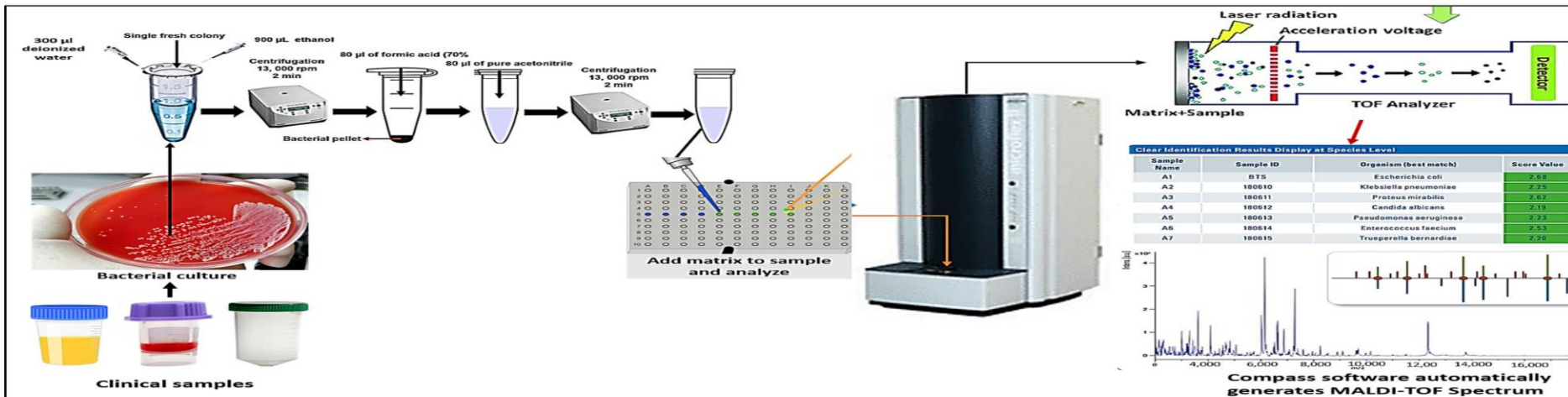
Among genomic methods (PCR) Polymerase Chain Reaction

1. These methods are more sensitive by several orders of magnitude than the protein-assays.
2. PCR gave no false positives. whereas , ELISA detected 2% false positives, specially in processed food samples .
3. Despite the high sensitivity, PCR assays cannot be applied to the hydrolyzed products such as syrup ,drinksand malt extracts for the determination of their
4. gluten content.



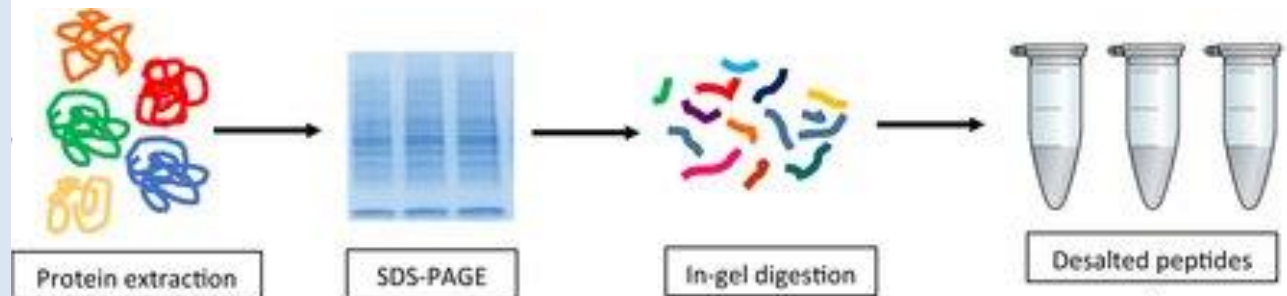
Among Proteomic methods Developments of testing

1. The relatively more direct and precise method for gluten detection and quantification is (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) (MALDI-TOF MS). *It can measure the protein and protein hydrolysate ranging in size from 1000 to 100,000 Daltons without a need for chromatographic purification.* Additionally, this technique allows reliable determination of protein levels as low as 0.01 mg/ml in the food samples . is a highly **sensitive** non-immunological approach for the **detection and quantification of gluten** contamination in food samples. However, its application requires **highly expensive specialized equipment**,

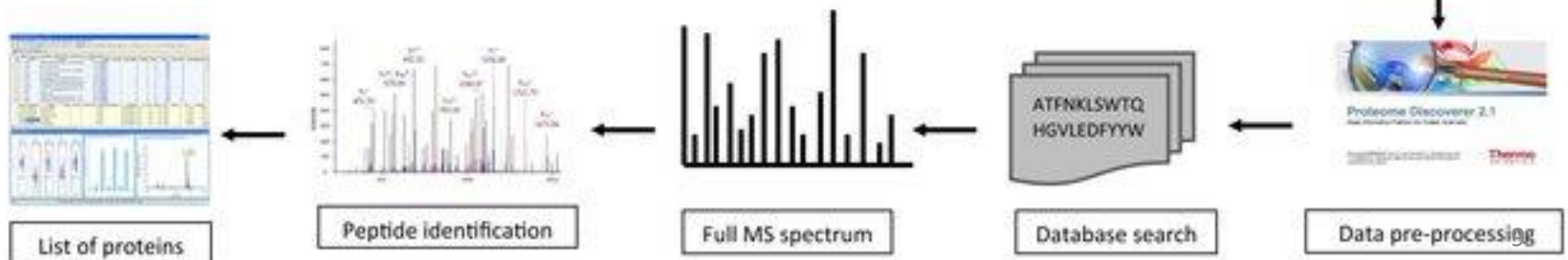


Among Proteomic methods Developments of testing

2- Coupling HPLC could overcome this limitation to tandem mass spectrometry (LC-MS/MS) .



LC-MS/MS analysis



Among Proteomic methods Developments of testing

4. Column chromatography is another method that has been used extensively for characterization, separation, and quantification of the cereal seed-storage proteins. Gel permeation (GP) chromatography, which separates proteins based on their molecular weights, and reverse-phase (RP) chromatography that separates proteins according to their hydrophobicities, are the most commonly used methods. These procedures have advantages in terms of speed (often 30 min runs) and detection capability, which is as low as 1-2 mg gluten. Although this method can be used to access gluten contamination reliably, it has the disadvantage of being unable to differentiate between gluten and non-gluten proteins in the complex food samples.

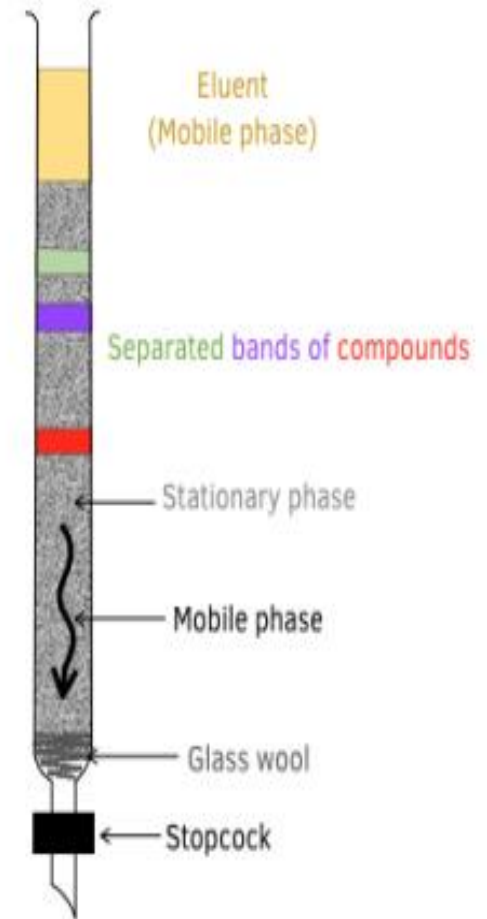
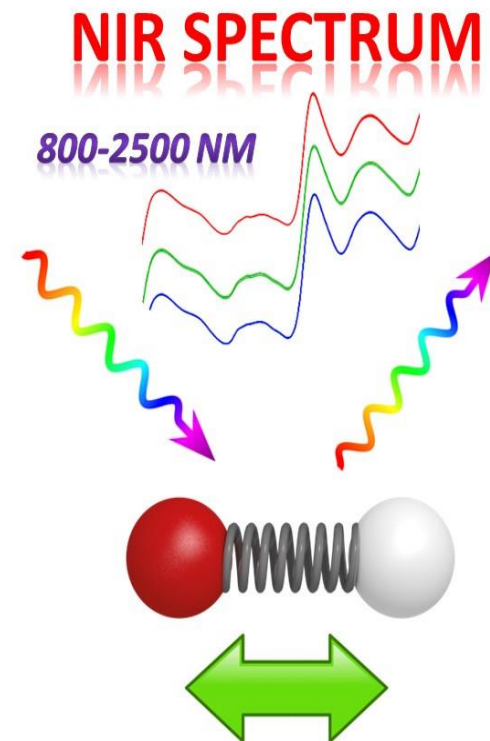


Fig 1. Column Chromatography Set-Up. The mobile phase flows downwards through the solid stationary phase.

Among Proteomic methods Developments of testing

5. the applicability of near-infrared (NIR) spectroscopy for the detection of gluten contamination in gluten-free products was proposed . For gluten detection and quantification, NIR spectroscopy was combined with chemometric techniques. However useful, this technique relies on the development of a suitable calibration model

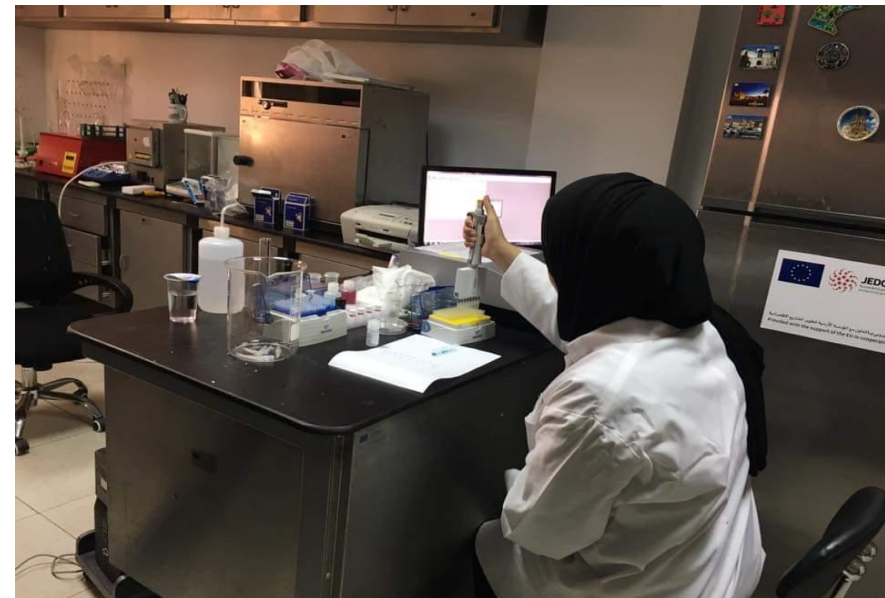


Among Immunological methods

- The more versatile and commonly accepted assays are immunological assays in particular ELISA.
- Owing to the sensitivity and speed of detection, the Codex Committee on Methods of Analysis and Sampling has endorsed these methods .
- Several variations of these methods have been developed
- Several antibodies (monoclonal and polyclonal) and a variety of commercial kits are available in the market to perform these assays .

The commonly used ELISA systems can be grossly divided into two categories:

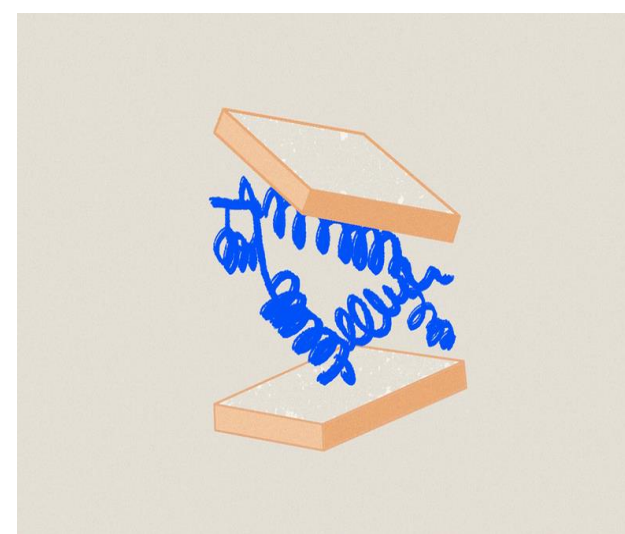
1. The sandwich ELISA
2. The competitive ELISA



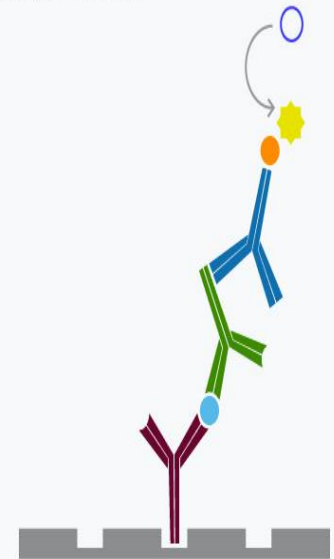
Among Immunological methods

1. The sandwich ELISA

- In the sandwich ELISA the antigen is sandwiched between two antibodies, one immobilized to the walls of the microtiter plate (capture antibody) and the other coupled with an enzyme (detection antibody).
- The sandwich ELISA is only suitable for **large antigens** because the antigen should have at least two separate epitopes to bind both antibodies.
- Thus, this ELISA system is **not an appropriate choice for partially hydrolyzed gluten samples** like in the sourdough products, malt, ...



SANDWICH ELISA



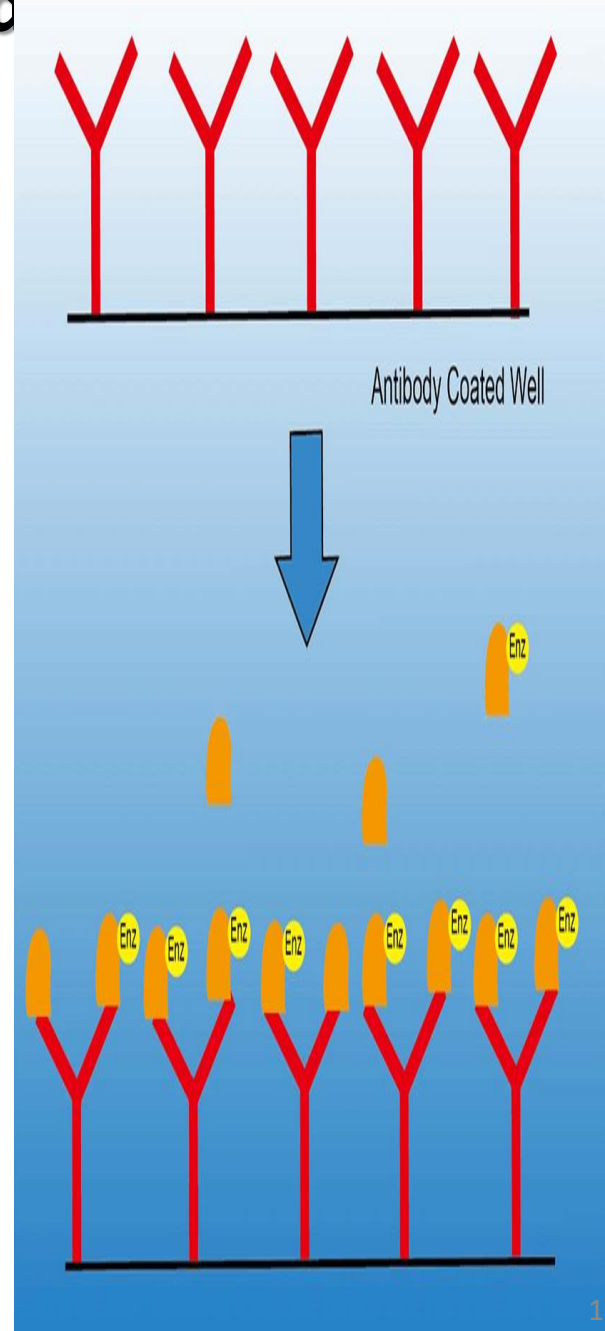
KEY



Among Immunological methods

2. The competitive ELISA

- Which is suitable for the detection of **small-sized antigens with a single epitope**.
- In this system, **labeled and unlabeled antigen** is applied to immobilized antibody, where they compete for the antibody binding sites.
- After washing out the unbound antigen, the quantity of the labeled antigen is determined by adding **the enzyme-substrate and measuring the intensity of the colored end product**, which corresponds with the **quantity of the labeled antigen**.



Among Immunological methods

The commonly used ELISA systems can be grossly divided into two categories:
the sandwich ELISA and the competitive ELISA .

The major problem associated with both of the ELISA systems is the determination of gluten contamination in heat-processed food samples, which cause conformational changes to the antigen masking or modifying the antibody recognition site(s)



Challenges in Gluten Detection

- Common Issues:

1. Accuracy,
2. sensitivity, and
3. interference from other food components and Contamination

- Improving Methods:

Ongoing research and development to enhance gluten detection accuracy.

- Approval of new methods and /or new instruments



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