



Application of Sandwich ELISA for Trace Level Detection of Gluten in Food Samples

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Food Laboratories Section



- Established in 1974, Food laboratories section is a governmental entity and involved in food testing in the country, operates three branches within the country.
- **ISO/IEC 17025:2017** accredited government laboratory with 72 accredited methods across broad field of food analytical sciences.
- The only National Laboratory responsible for food safety control within the state of Qatar.
- 50th years as collaborating centre by international agencies.

ISO Accreditation

ANAB: L2412


ANSI National Accreditation Board

CERTIFICATE OF ACCREDITATION

The ANSI National Accreditation Board
Hereby attests that

Food Laboratories Section
National Health Laboratories (NHL)
Messimer, Zone 56, Building 56110168
Ministry of Public Health
Doha City, State of Qatar

Fulfills the requirements of

ISO/IEC 17025:2017
and
AOAC International Guidelines for Laboratories Performing
Microbiological and Chemical Analysis of Food, Dietary Supplements and
Pharmaceuticals (2018)

In the field of
TESTING

This certificate is valid only when accompanied by a current scope of accreditation document.
The current scope of accreditation can be verified at www.anab.org.


Jason Stine, Vice President
Expiry Date: 02 April 2027
Certificate Number: L2412





This laboratory is accredited in accordance with the recognized International Standard ISO/IEC 17025:2017.
This accreditation demonstrates technical competence for a defined scope and the operation of a laboratory
quality management system (refer to joint ISO-ILAC-IAF Communiqué dated April 2017).

Analytical Units



Microbiology



Molecular biology



Chemistry



Radiation

Microbiology Unit

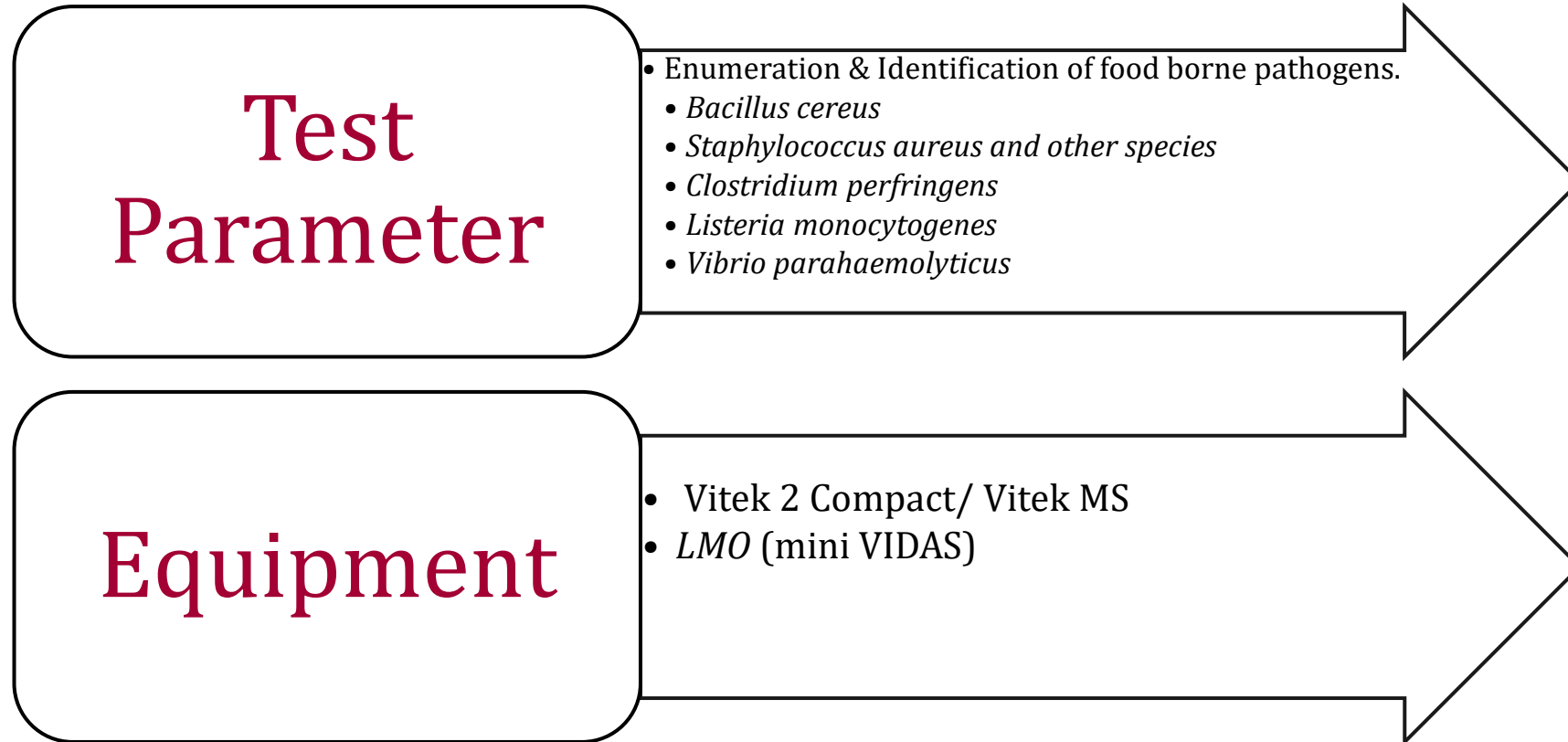
Test Parameter

- Enumeration of food spoilage / food quality indicator microorganisms (Enterobacteriaceae, Coliforms, Fecal Coliforms, *Escherichia coli*)
- Yeasts & Molds

Equipment

- Colony Counter
- Vitek MS (identification)

Microbiology Unit



Microbiology Unit

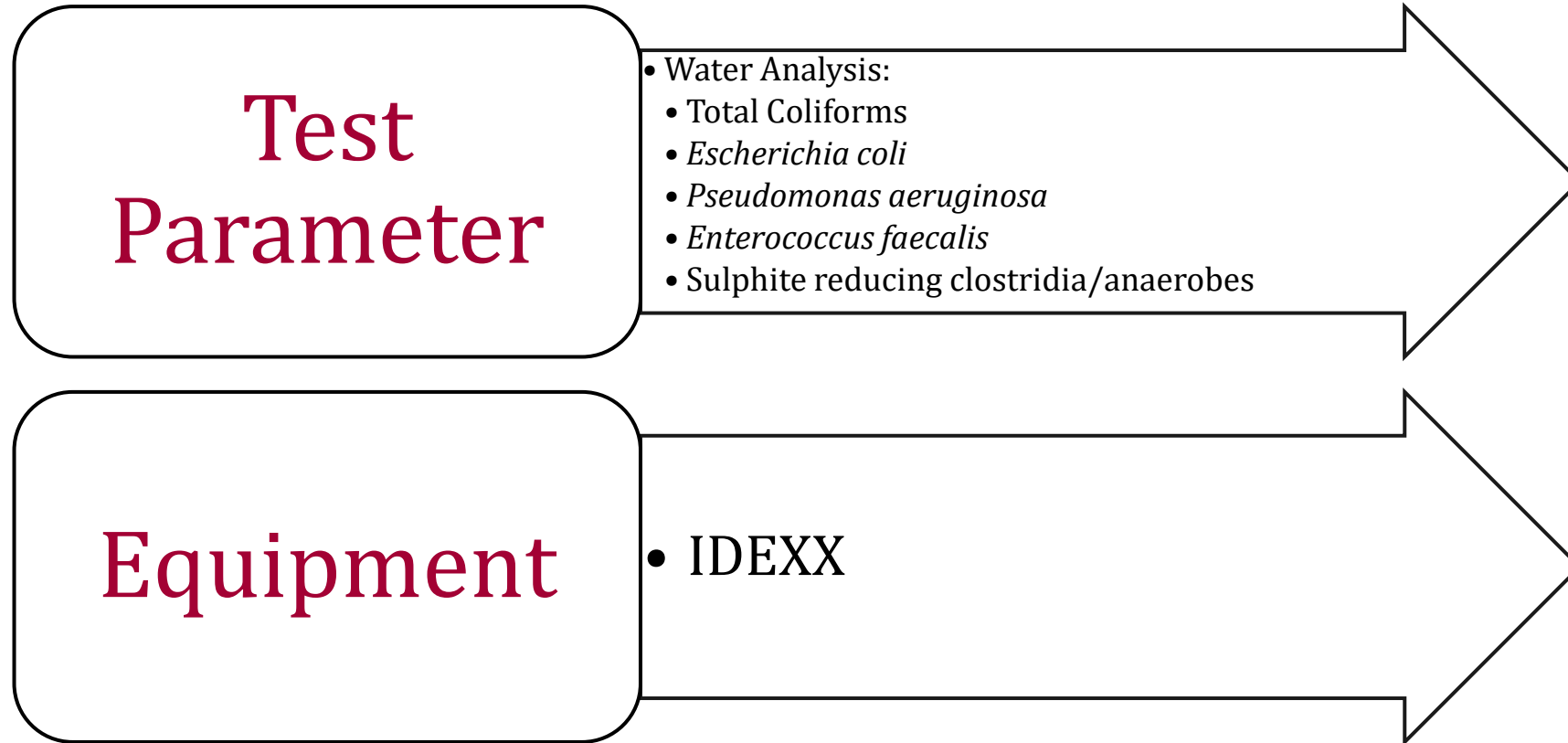
Test Parameter

- Detection & Identification of food borne pathogens:
- *Salmonella* spp.
- *Escherichia coli* O157:H7
- *Listeria* spp., LMO
- *Vibrio* spp., *Vibrio parahaemolyticus*
- *Campylobacter jejunii*,
- *Cronobacter sakazakii*
- *Pseudomonas aeruginosa*

Equipment

- Vitek 2 Compact
- Vitek MS
- *Listeria* spp. & LMO (mini VIDAS)

Microbiology Unit



Molecular Biology Unit

Test Parameter

- Detection of Pathogens:
 - *Salmonella* spp.
 - LMO
 - *Campylobacter jejunii*
 - *Escherichia coli* O157:H7
 - *Shigilla* spp.
 - *Yerisinia enterocolotica*

Equipment

- RT-PCR

Molecular Biology Unit

**Test
Parameter**

- DNA Detection:
 - Pork
 - Horse
 - Cat & Dogs

Equipment

- RT-PCR

Molecular Biology Unit

Test Parameter

Detection of Food Viruses:

- Noroviruses GI & GII
- Hepatitis A & B
- Hepatitis E

Equipment

- RT-PCR

Molecular Biology Unit

Test Parameter

Detection of Food Parasites:

- Detection of *Cryptosporidium* spp.
- Detection of *Entamoeba histolytica*
- Detection of *Giardia intestinalis*

Equipment

- RT-PCR

Radiation Unit

Test Parameter

- All Alpha (e.g.: Uranium Isotopes, Ra-226, Po-210 etc.,)
- Beta emitters (Ra-228, Sr-90, H-3 etc.,)
- All Nuclides for Gamma (Ex: Cs-137, Cs-134, K-40, Am-241, Co-60, Co-57, etc.,)
- Cs-137 (Screening)

Equipment

- Liquid Scintillation Counter (LSC) for Gross Alpha/Beta
- Gamma Spectroscopy System (HPGe Detector)
- Alpha Spectroscopy System
- Gamma Spectroscopy System (NaI Detector)

Chemistry Unit

Test Parameter

- **General Chemistry:**
 - Proximate Composition: Moisture, Ash, Fat, Protein, Fiber content & Carbohydrates.
- pH
- Fatty Acid Methyl Esters (FAME)
- Dairy Products: (Fat, Protein, TS, MSNF)

Equipment

- KJELDTEC
- pH meter
- HPLC
- Milkoscan

Chemistry Unit

**Test
Parameter**

- **Food Additives:**
 - Preservatives (Benzoic & Sorbic acid)
 - Artificial Sweeteners (Aspartame & Saccharin)
 - Synthetic colors & Illegal Dyes
 - Flavor enhancers (MSG)

Equipment

- UPLC
- UHPLC
- GC

Chemistry Unit

Test Parameter

- Veterinary Drugs Residues
- Pesticides Residues
- Mycotoxins (Aflatoxins B1, B2, G1, G2)
- Minerals & Heavy Metals

Equipment

- LCMSMS
- GC-MS/MS
- UHPLC
- ICP-OE&ICP-MS

Chemistry Unit

Test Parameter

- Water Analysis
 - Physical Tests (Colour, Turbidity, pH, conductivity, Alkalinity)
 - Chemical Tests (Anions, Cations, traces elements, Trihalomethanes)

Equipment

- Colorimeter
- Autotitrator
- HS-GC-MS
- ICPMS

Importance of Gluten Detection

- Gluten, a complex mixture of proteins found in wheat, barley, and rye, poses a significant concern for individuals with gluten-related disorders.
- Accurate detection of gluten in food products is crucial to ensure compliance with regulatory standards and to safeguard the health of consumers.



Importance of Gluten Detection

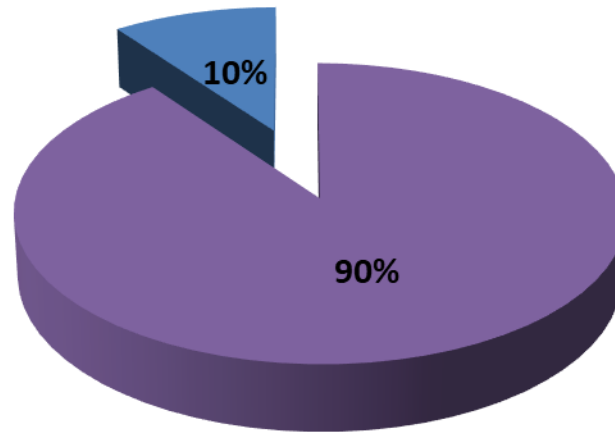
- Gluten sensitivity and celiac disease are on the rise globally, necessitating stringent gluten labeling laws and accurate detection methods.
- Reliable detection methods are essential for food manufacturers to ensure product safety and compliance with regulations.



Prevalence of Food Allergies

90%
Peanut
Tree Nuts
Milk
Egg
Soy
Fish
Shellfish
Wheat

10%
Hundreds of
Others



■ Top Eight

■ Others

The History of Gluten Analysis in Food

- In the early days, gluten analysis relied on basic chemical methods, such as protein precipitation and gravimetric determination.
- Time-consuming, lacked specificity, and could not differentiate between gluten from different sources.

Development of Immunoassays

The introduction of immunoassays revolutionized gluten analysis. *The enzyme-linked immunosorbent assay (ELISA)* emerged as a powerful tool for detecting and quantifying gluten in food samples. Early ELISA methods utilized polyclonal antibodies and provided a significant improvement in specificity and sensitivity compared to traditional methods.

R5 ELISA Method

In the 1990s, the *R5 ELISA method* was developed by the R-Biopharm company in collaboration with the Prolamin Working Group.

- Monoclonal antibodies specific to gliadin, a component of gluten.
- Adopted as a reference method for gluten analysis and endorsed by organizations such as the Codex Alimentarius Commission.

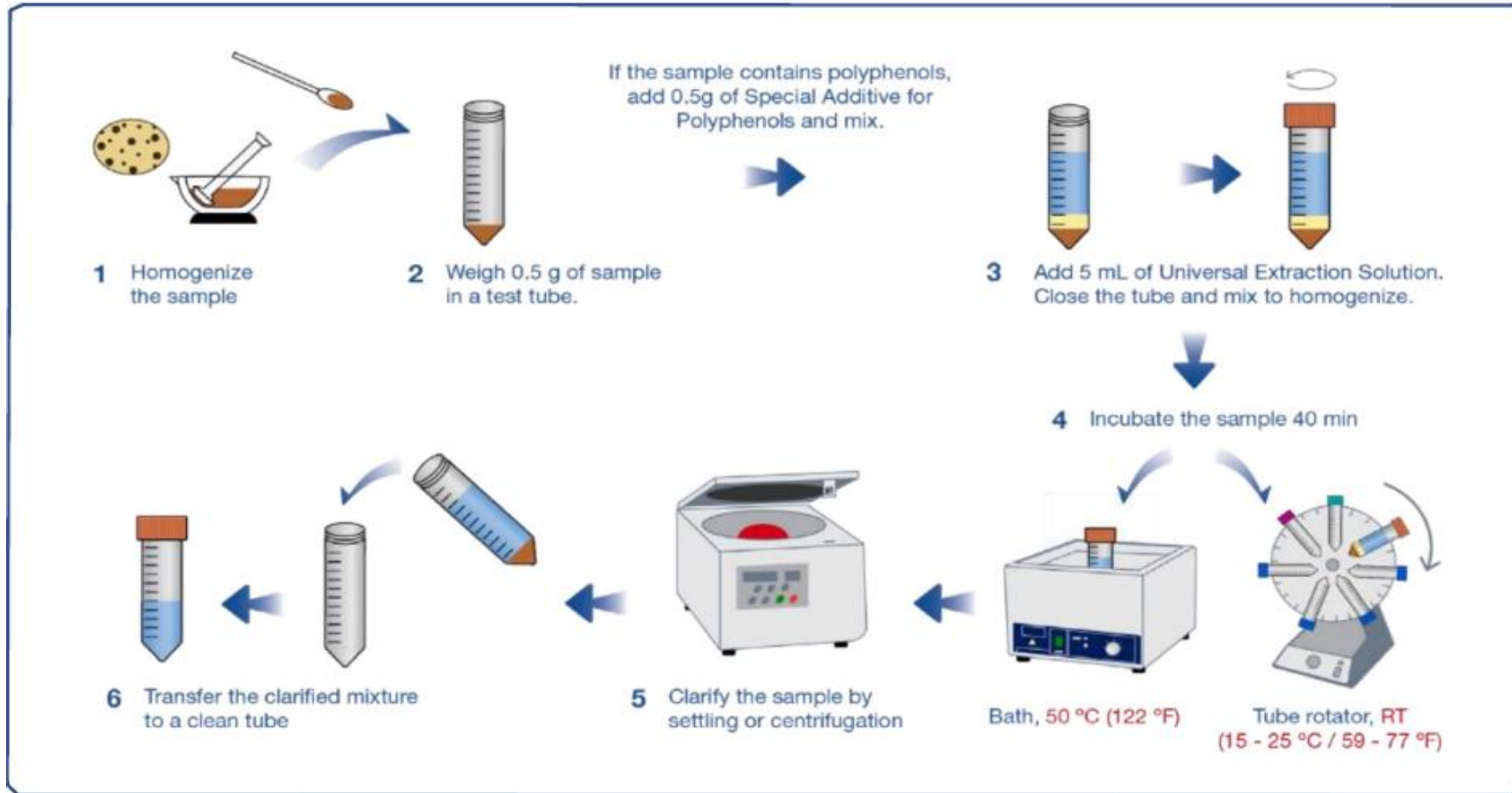
Understanding Sandwich ELISA

Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) is a sensitive and specific analytical technique.

The target protein is sandwiched between two antibodies – a capture antibody immobilized on the plate surface and a detection antibody conjugated with an enzyme.

The basic steps include Coating, Blocking, Incubation with sample, Washing, Incubation with detection antibody, Washing, and Detection.

Application of Sandwich ELISA for Gluten Detection



Application of Sandwich ELISA for Gluten Detection



SAMPLE PREPARATION:
EXTRACTION AND PURIFICATION
OF GLUTEN FROM FOOD
SAMPLES



CHOICE OF ANTIBODIES:
SELECTION OF HIGHLY SPECIFIC
ANTIBODIES TARGETING
GLUTEN PROTEINS



CALIBRATION CURVE: STANDARD
CURVES GENERATED USING
KNOWN GLUTEN
CONCENTRATIONS FOR
QUANTIFICATION



DATA ANALYSIS: CALCULATION
OF GLUTEN CONCENTRATIONS
BASED ON ABSORBANCE
READINGS

Advantages of Sandwich ELISA

High sensitivity
and specificity

Ability to detect
trace levels of
target analyte

Suitable for
complex sample
matrices

Quantitative
analysis
capability

Challenges in Gluten Detection

Gluten's presence in various food matrices

Need for high sensitivity technique to detect trace levels

Cross-reactivity with other proteins

Regulatory requirements for gluten-free labeling

Conclusion

- ✓ Robust and reliable method for trace level detection of gluten in food samples.
- ✓ Advancements in gluten detection technologies will further enhance food safety and meet the needs of consumers with gluten-related disorders.
- ✓ Zero tolerance of allergen content in food (labelling requirement) depends highly on the specificity of the test method.
- ✓ According to the Codex Alimentarius Commission and the EC Regulation 41/2009 on the composition and labeling of foodstuffs suitable for people intolerant to gluten, food can be considered "gluten-free" if its gluten content does not exceed 20 parts per million (ppm).
- ✓ The high demand on gluten free food products due to conscious on allergic reaction necessitated the development of method to quantify the trace level of gluten.
- ✓ The efforts of national & international organizations to boost the customer confidence in the safety & reliability of food is thus guaranteed.



THANK YOU