
IN THE NAME OF GOD

Topic:

Detection of Escherichia coli O157:H7 Using Automated Immunomagnetic Separation and Enzyme-Based Colorimetric Assay

Presented by:
Khatereh Sadat Ekhteraei

MSc student of medical bacteriology, Isfahan University of Medical Sciences

Outline

Introduction
Characteristics of magnetic particles
Application of magnetic particles
Immunomagnetic separation system
Enzyme-Based Colorimetric Assay
Materials and methods
Isolation pathogen bacteria in food
Isolation DNA and Protein in bacteria
Result
Conclusion

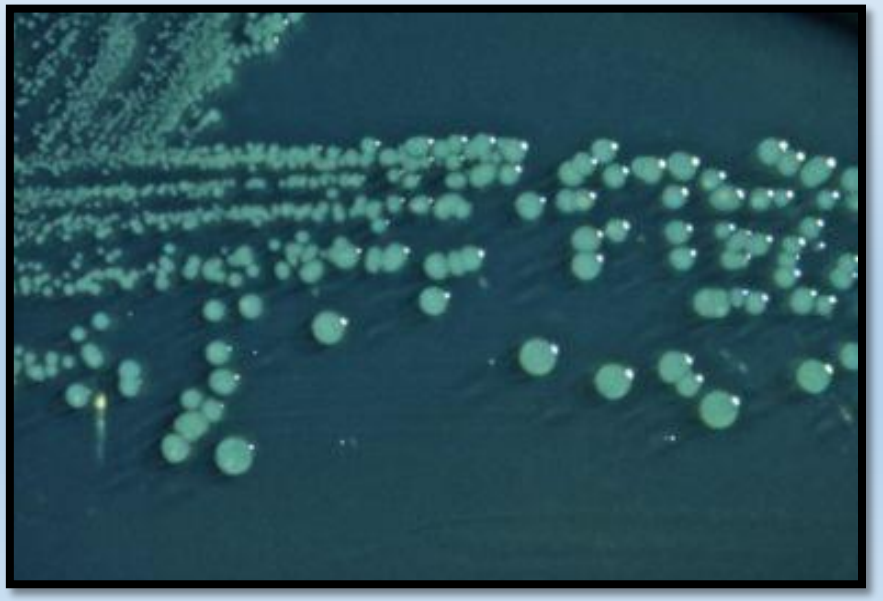
Introduction

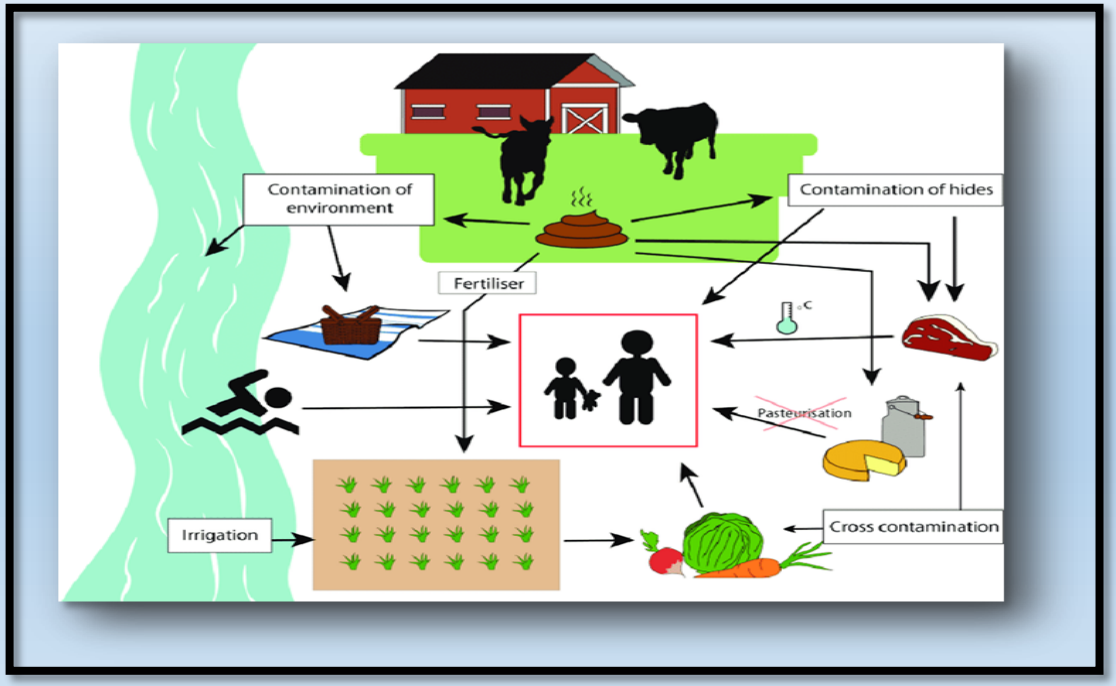
Infectious diarrhea is one of the major health problems worldwide.

Enterohemorrhagic Escherichia coli O157:H7 (E. coli O157:H7) is one of the major foodborne pathogens producing cytotoxins such as verotoxin and shiga toxin.

According to the **Center for Disease Control and Prevention** (CDC), transmission of E. coli O157:H7 causing food poisoning is primarily occurred by contaminated food and water.

The **main cause** of the disease is contaminated food such as water, milk, vegetables, fruits, salads and ice cream.



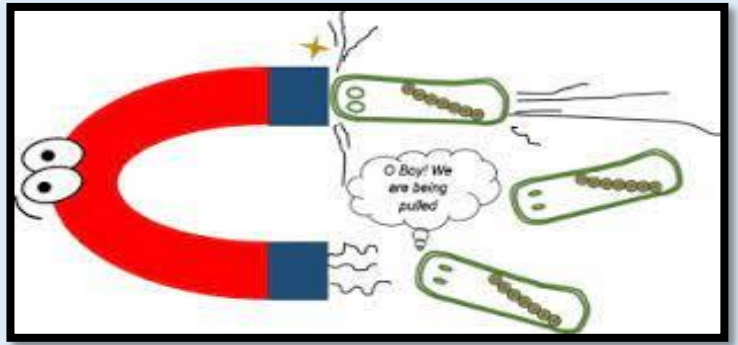


Traditional methods for detecting foodborne bacteria can take a few days because these include long enrichment steps and consist of laborious experimental steps for confirming the bacteria cells and require a trained person to perform the test.

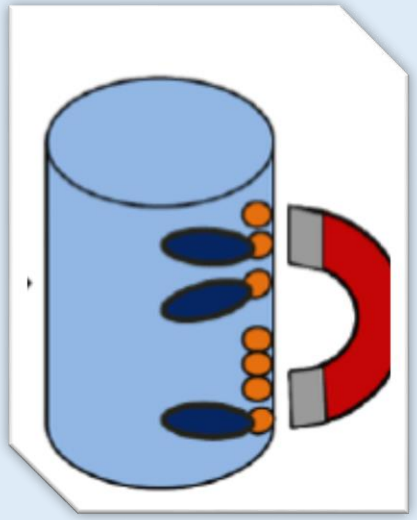
Therefore, there is a crucial issue for the development of a **simple method to provide easy, fast and automatic systems** for separating and detecting the foodborne bacteria from food samples.

Magnetic nanoparticles are a class of nanoparticle that can be manipulated using magnetic fields.

Nanoparticles are smaller than 1 micrometer in diameter (typically 1–100 nanometers).



Characteristics of magnetic particles



- Stable particles that do not change under the influence of reagents.
- There is no specific particle for different samples, only surface markers change.
- The turbidity or persistence of the sample has no effect on the magnetic properties of these particles.
- Using a magnetic field, the performance of these particles can be slightly affected.

Application

Common technique to separate:

- Microorganism such as bacteria ,virus , fungus and parasite
- Cellular organs
- Biologically active compounds are nucleic acid proteins
- Because of the inherent ability of magnets Magnetic separation is a standardized step in various fields such as tissue engineering , medicine and basic research

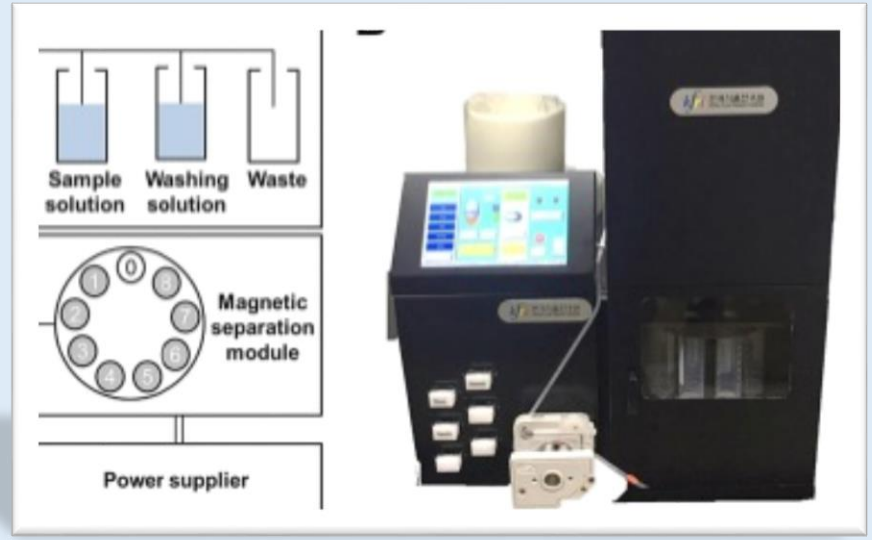
Immunomagnetic separation

Immunomagnetic separation (IMS) is effective for identifying bacteria from foods due to selective separating target molecules from a heterogeneous matrix.

A target-specific antibody on the surface of a magnetic bead is a crucial factor for increasing the yield of selective separation and the concentration of the antigenic target.

IMS method offers some advantages in the food safety field including high selectivity and rapidity for detecting a pathogens.

In **this study**, we present an **automated IMS system combined with an enzyme-based colorimetric** assay for the rapid detection of pathogenic E. coli O157:H7 from food samples.

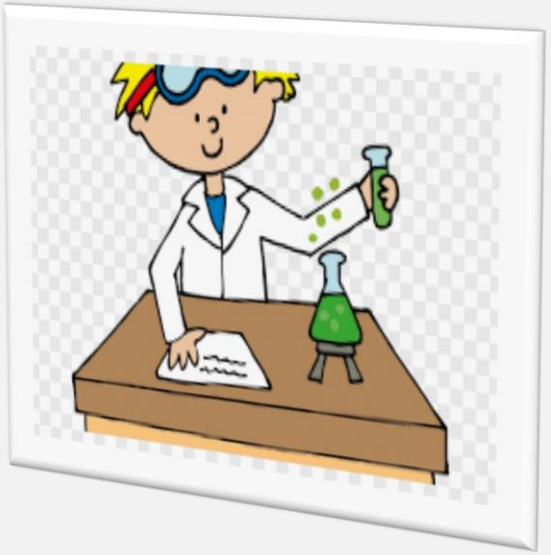


Materials

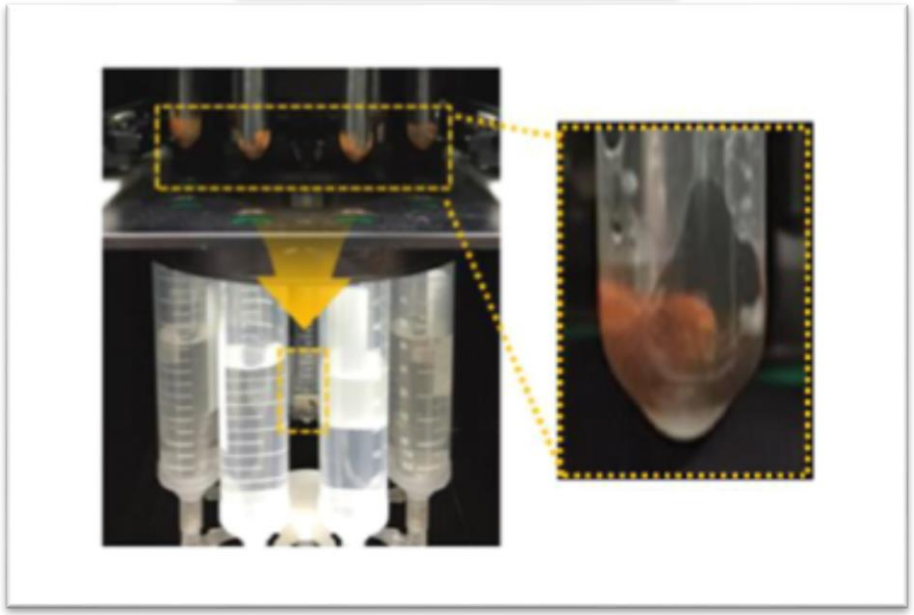
Chlorophenol red -galactopyranoside (CPRG), Buffered peptone water (BPW), phosphate buffered saline (PBS, pH 7.4) and Tween-20 , Bacterial Protein Extraction Reagent (B-PER), Sorbitol MacConkey agar (SMAC), Tryptic soy broth (TSB),cefixime-tellurite supplement,magnetic bead Falcon tissue cultural treated-96 well microplate were purchased.

Methods

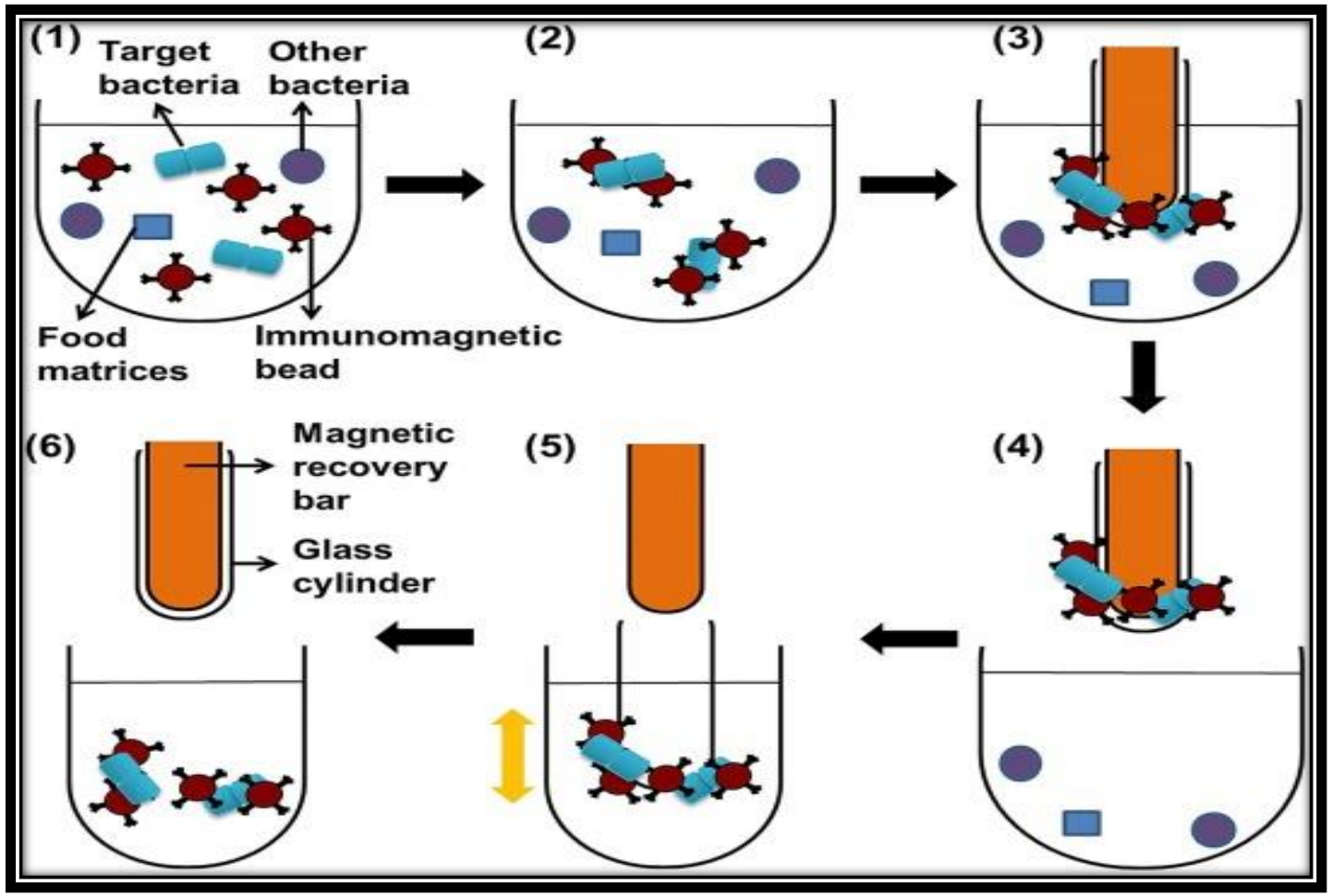
- **Bacteria Enrichment Culture and Immunomagnetic Bead Reaction**
- **Immunomagnetic Separation and Concentrationof E. coli O157:H7 in Milk Samples**
- **Enzyme-Based Colorimetric Detection of E. coli O157:H7**



Bacteria isolation

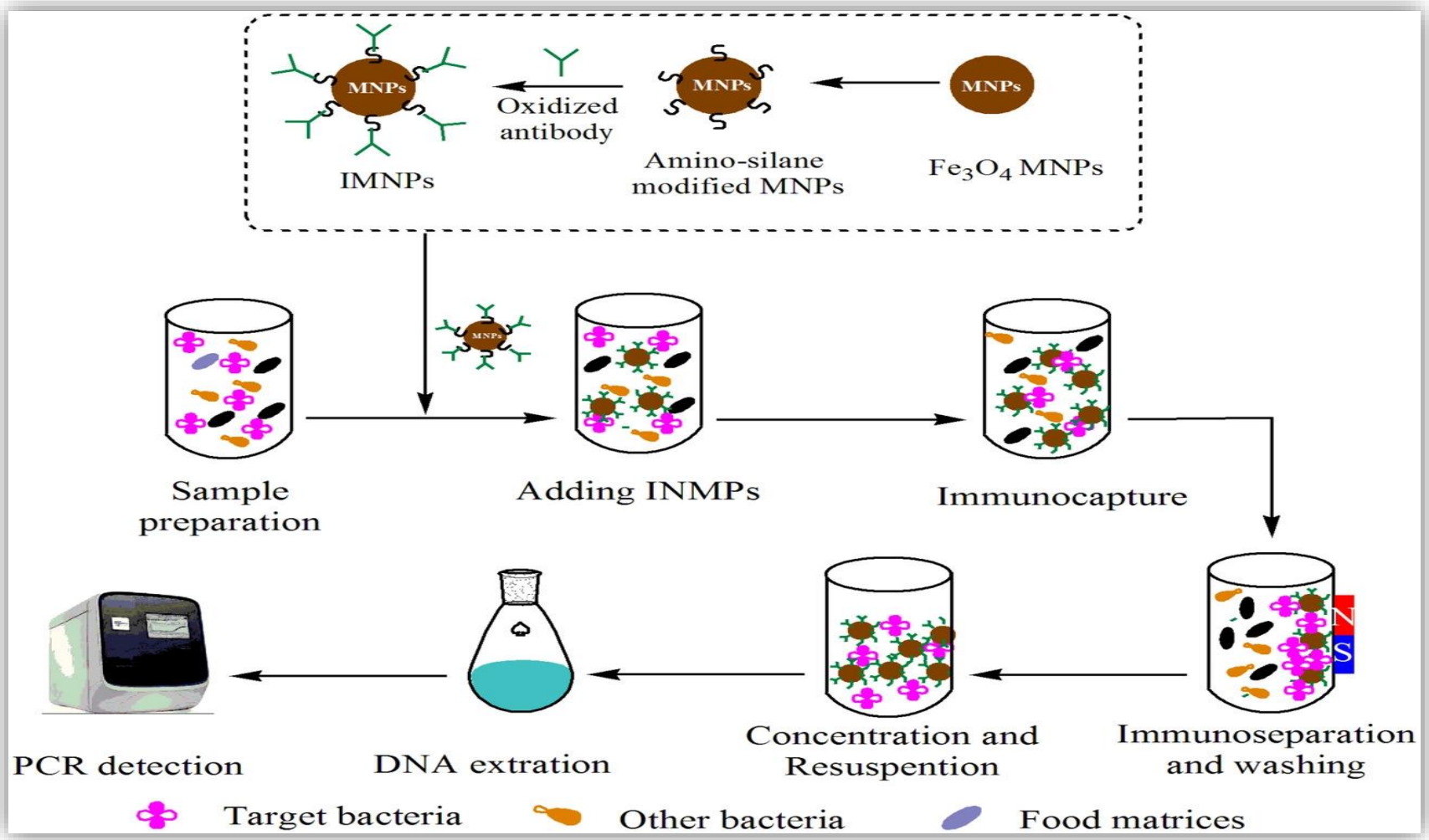


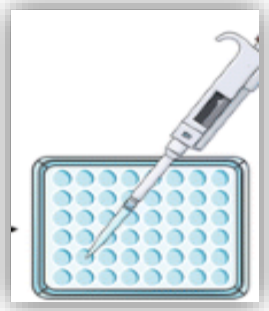
Automated IMS system

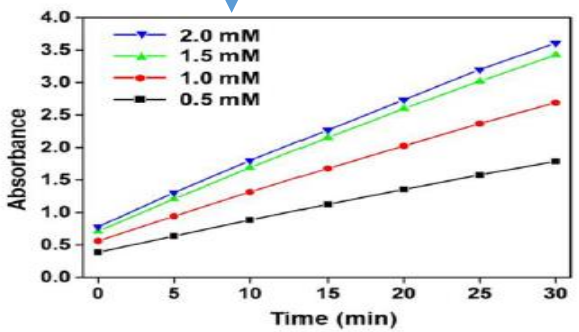


Immunomagnetic separation from sample matrix

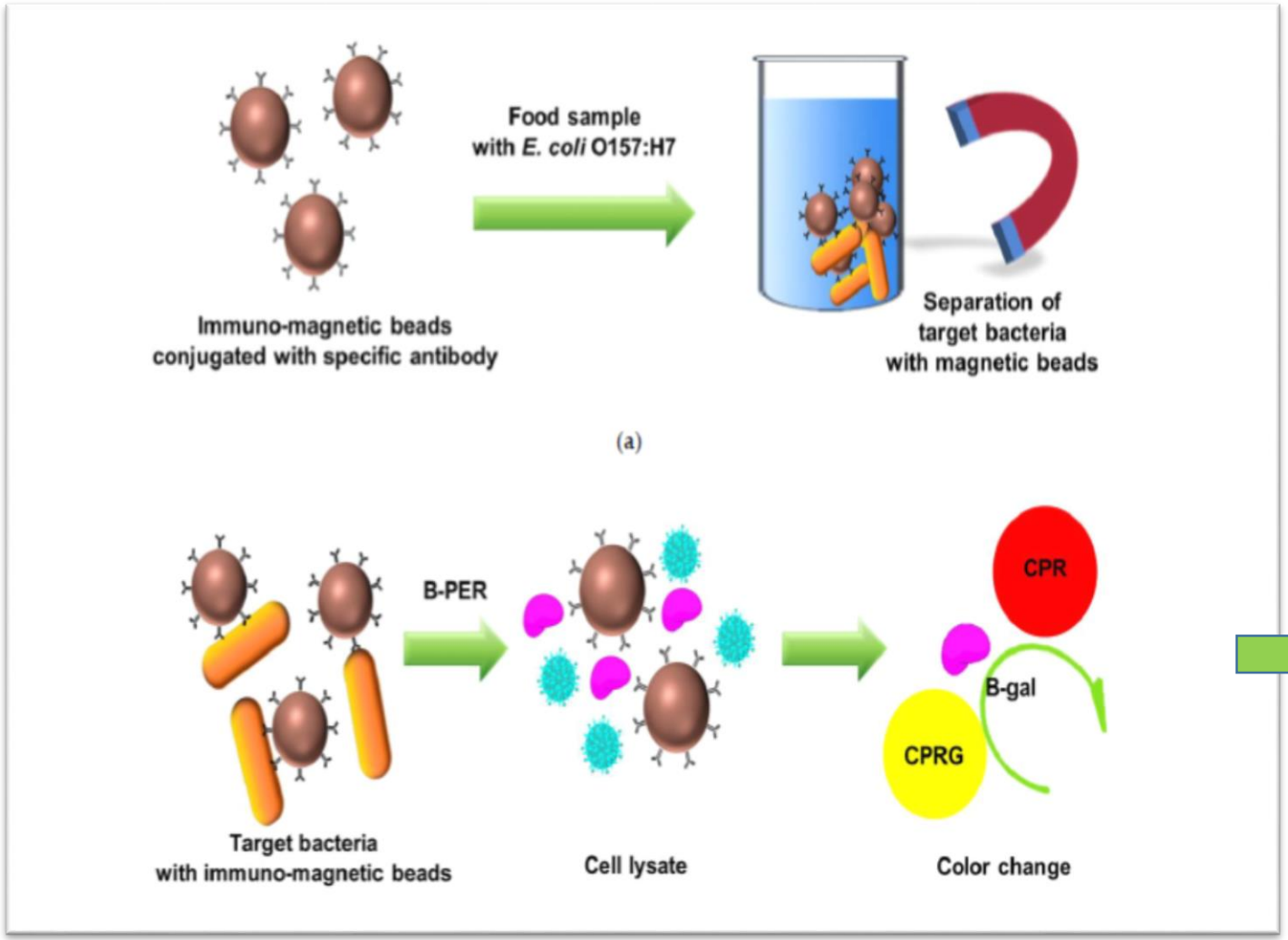
DNA isolation

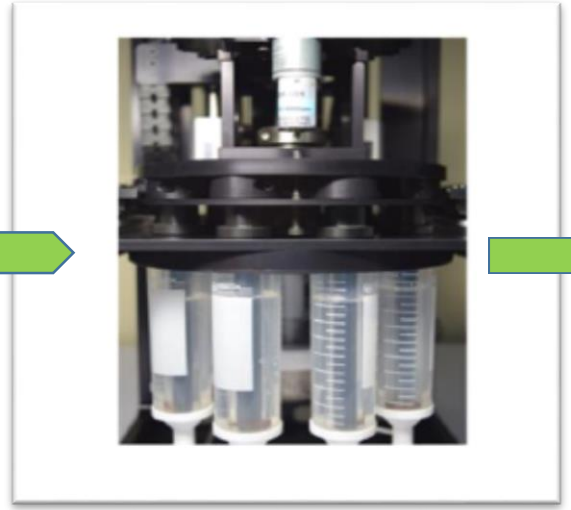




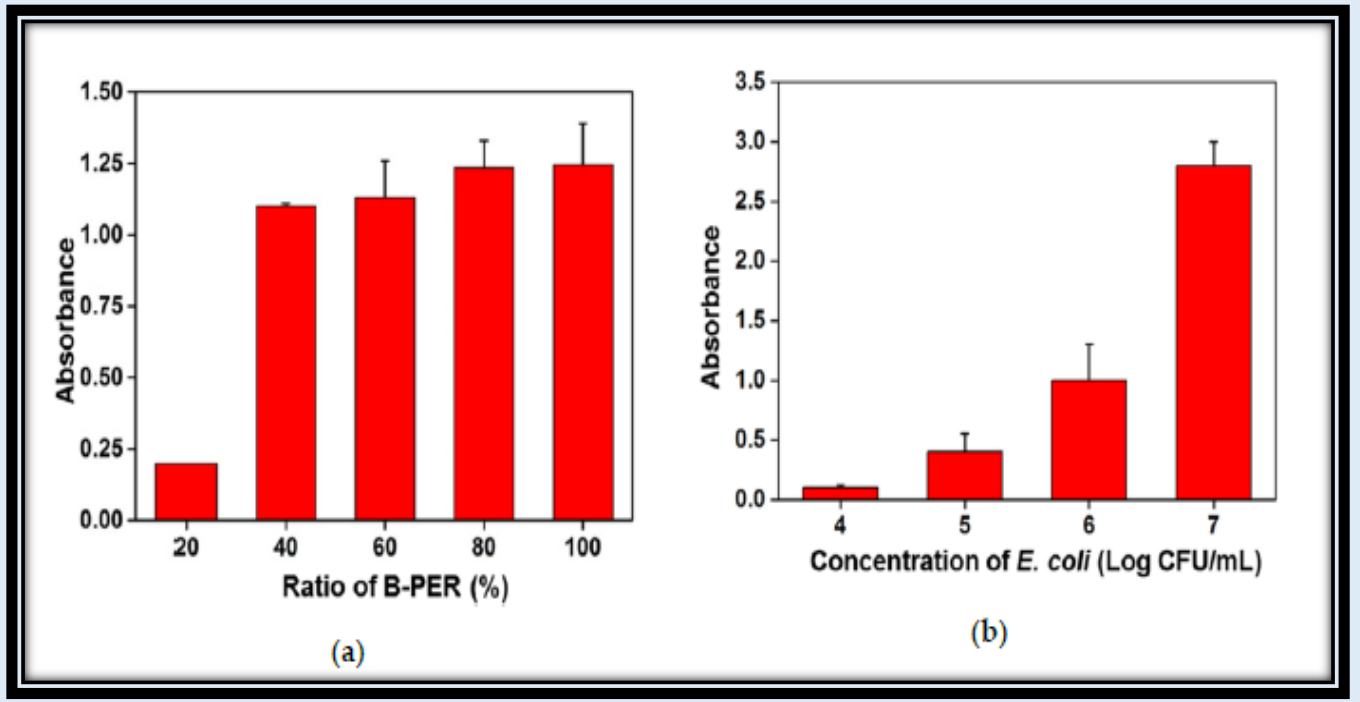


Protein isolation



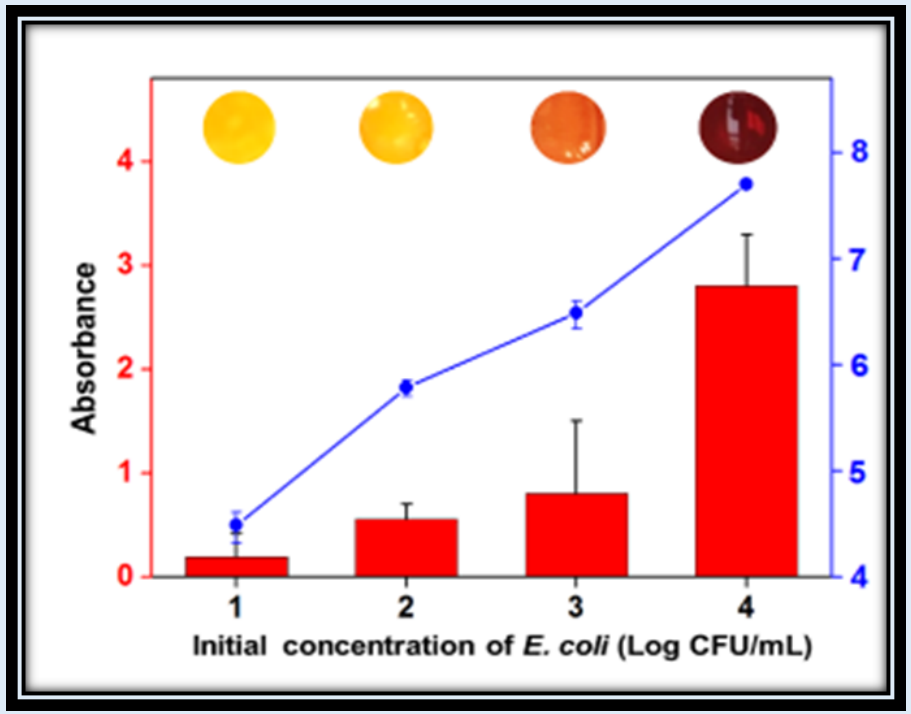


Result



Optimization of enzymatic colorimetric change reactions for the sensitive detection of target bacteria:
(a) Colorimetric response depending on the mixing ratio between B-PER and 5 mM PBS for the lysis of target bacteria;
(b) Comparison of absorbance in the range 10^4 to 10^7 CFU/ml of target bacteria under the optimized experimental conditions

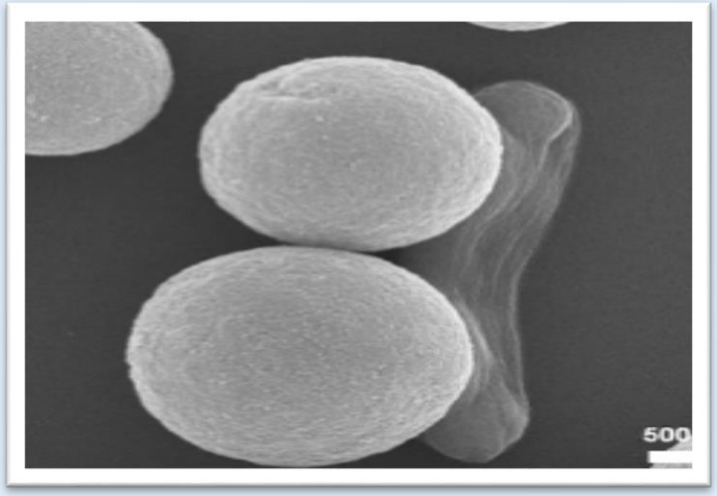
Specific colorimetric response on target E.coli after immunomagnetic separation and enzymatic reaction



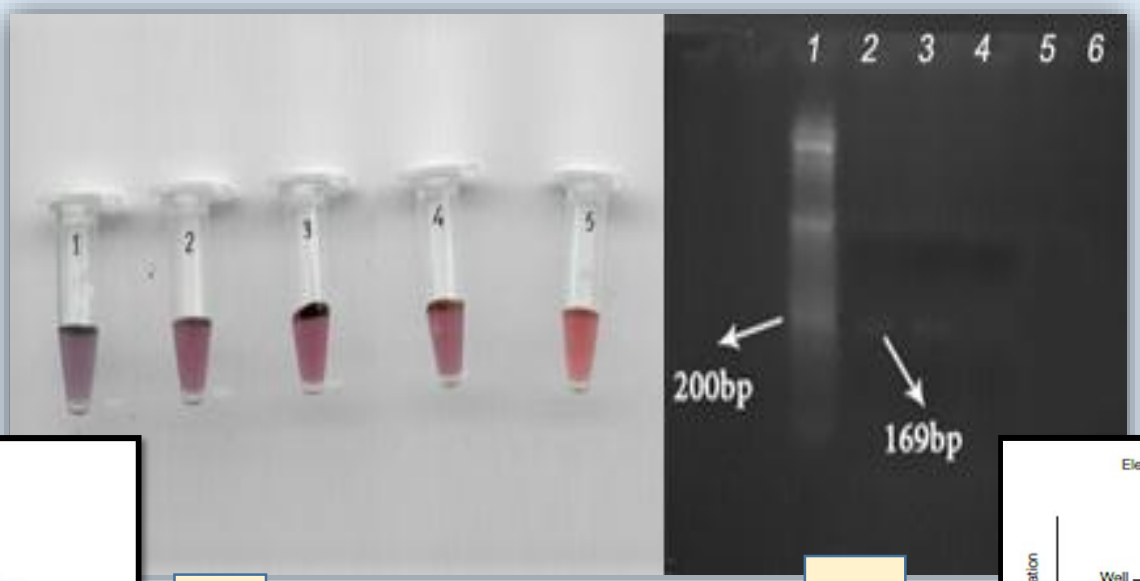
Bacterial Strains	Colorimetric Change	Absorbance Change	Final Reaction Solution Color
E. coli O157:H7	Yes	Yes	
S. enterica	No	No	
S. aureus	No	No	

Recovery concentration of bacteria evaluated by enzymatic reaction

Representative SEM image of target E. coli O157:H7 captured by immunomagnetic beads from milk samples after the automated IMS process

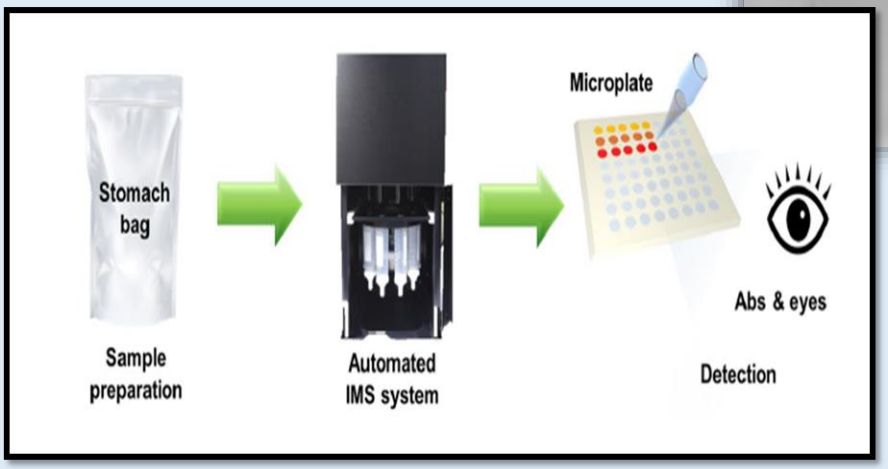


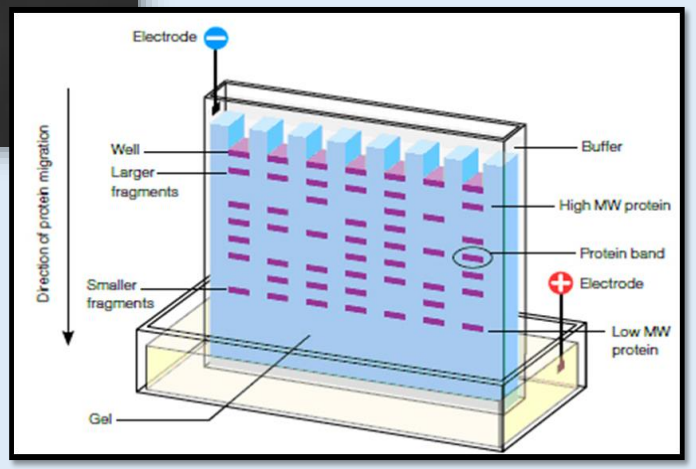
A)Electrophoresis image
B)Colorimetric test tubes



A

B



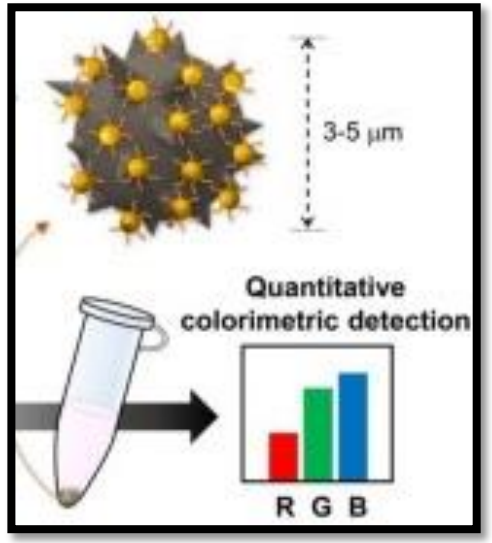


Conclusions

Shigellosis is the clinical presentation of Shigella infection. Disease is transmitted through the fecal-oral route, with an infectious dose of only 10–100 organisms.
Early detection of this disease is essential.

Comparison between traditional methods and modern methods

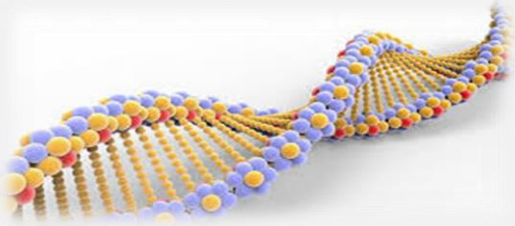
Traditional test	IMS with Colorimetric
Slow,complicated	Rapid,Easy
Detection of bacteria in food in a few days	Detection of bacteria in food take less than 3 h
require a trained person to perform the test	High sensitivity of colorimetric test by the naked eye
Requires confirmatory biochemical tests	collect the target bacteria from a large volume of the food sample



In this study, proposed a **rapid** and **easy** detection method for E. coli O157:H7 using an automated IMS system and enzyme-based colorimetric assay.

The **advantage** of the combined process is the detection of target bacteria in a quantitative manner through numerical values from absorbance measuring and in a qualitative manner by color information seen by the **naked eye**.

The **remarkable** thing about DNA magnetic purification is that nucleic acids can be separated directly from raw materials such as blood, homogenized tissue ,water and culture medium.



As a **result**, we anticipate that the designed process will be a promising on-site detection method such as paper-based strip sensors to identify the pathogenic bacteria in foods.

Main article:

sensors

MDPI

Article

Detection of *Escherichia coli* O157:H7 Using Automated Immunomagnetic Separation and Enzyme-Based Colorimetric Assay

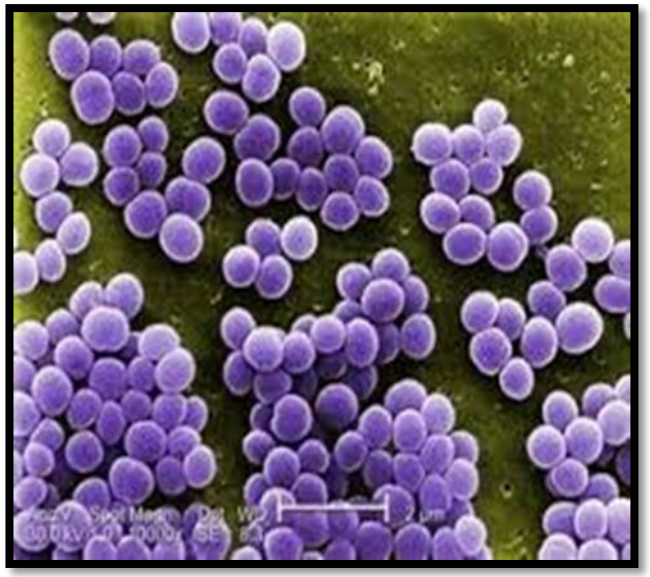
Ji Young Park [1], Kisang Park [2,3], Gyeongsik Ok [2], Hyun-Joo Chang [2], Tae Jung Park [1], Sung-Wook Choi [2] and Min-Cheol Lim [2,*]

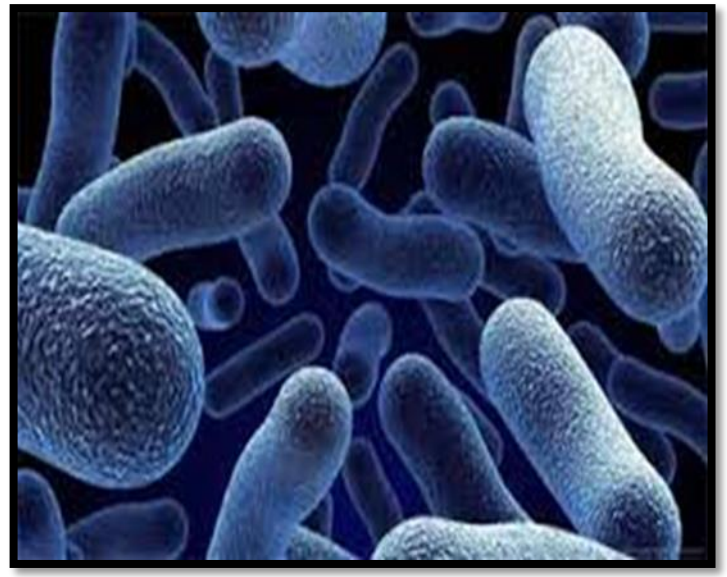
1 Department of Chemistry, Chung-Ang University, Seoul 06974, Korea; jrorangece@naver.com (J.Y.P.); tjpark@cau.ac.kr (T.J.P.)
2 Research Group of Consumer Safety, Korea Food Research Institute (KFRI), Jeollabuk-do 55365, Korea; 07938@kfri.re.kr (K.P.); gsok@kfri.re.kr (G.O.); hjchang@kfri.re.kr (H.-J.C.); swchoi@kfri.re.kr (S.-W.C.)
3 Department of Molecular Science and Technology, Ajou University, Gyeonggi-do 16499, Korea
* Correspondence: mclim@kfri.re.kr

Received: 4 February 2020; Accepted: 3 March 2020; Published: 4 March 2020

check for updates

Abstract: The food industry requires rapid and simple detection methods for preventing harm from pathogenic bacteria. Until now, various technologies used to detect foodborne bacteria were time-consuming and laborious. Therefore, we have developed an automated immunomagnetic separation combined with a colorimetric assay for the rapid detection of *E. coli* O157:H7 in food samples. The colorimetric detection method using enzymatic reaction is fascinating because of its simplicity and rapidity and does not need sophisticated devices. Moreover, the proposed procedures for the detection of bacteria in food take less than 3 h including pre-enrichment, separation and detection steps. First, target-specific immunomagnetic beads were introduced to contaminated milk in a pre-enrichment step. Second, the pre-enriched sample solution containing target bacteria bound





Thank you for your attention!

Any questions?