DEVELOPMENT OF IMMUNOAFFINITY BASED DETECTION PLATFORMS FOR FOOD PATHOGENS

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

DİLEK ÇAM

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
IN
BIOLOGY

MARCH 2016

Approval of the thesis:

DEVELOPMENT OF IMMUNOAFFINITY BASED DETECTION PLATFORMS FOR FOOD PATHOGENS

submitted by **DİLEK ÇAM** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Biology Department, Middle East Technical University** by**,**

Prof. Dr. Gülbin Dural Ünver _____
Dean, Graduate School of **Natural and Applied Sciences**

Prof. Dr. Orhan Adalı _____
Head of Department, **Biology**

Prof. Dr. Hüseyin Avni Öktem _____
Supervisor, **Biology Dept., METU**

Examining Committee Members:

Prof. Dr. Meral Yücel _____
Biology Dept., METU

Prof. Dr. Hüseyin Avni Öktem _____
Biology Dept., METU

Prof. Dr. Füsun Eyidoğan _____
Science Education Dept., Başkent University

Assoc. Prof. Dr. Remziye Yılmaz _____
Food Engineering Dept., Hacettepe University

Assoc. Prof. Dr. M. Cengiz Baloğlu _____
Genetic and Bioengineering Dept., Kastamonu University

Date: 30.03.2016

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name **:** Dilek Çam

Signature **:**

ABSTRACT

DEVELOPMENT OF IMMUNOAFFINITY BASED DETECTION PLATFORMS FOR FOOD PATHOGENS

Çam, Dilek

Ph.D., Department of Biology

Supervisor: Prof. Dr. Hüseyin Avni Öktem

March 2016, 164 pages

Salmonella and *E.coli*, food pathogens, are among the very important pathogens threating the health. Rapid and easy detection of these pathogens is crucial. In this context, lateral flow assay (LFA) platform prepared by gold nanoparticles (GNPs) based on antibody for Salmonella and *E.coli* O157:H7 was developed in the first part of this study. In second part, single chain variable fragments (scFv) and SNAP-Tagged full IgG (fusion protein) of se155-4 antibody specific to Salmonella were genetically produced. Target capturing potential of the antibodies was examined by conventional and immunomagnetic ELISA.

HF180 and 36nm GNPs were ideal membrane and dimension, respectively in the developed LFA. The best recognized species were *S. enteritidis* and *S. infantis. E.coli* were also specifically captured by developed strips. $5x10^5$ *S. typhimurium* and $3x10^4$ *E.coli* cells were determined as limit of detection by strips.

scFv captured $2.8x10^4$ and $2.8x10^5$ target cells alone and in bacterial mixture, respectively. To our knowledge, recognition of whole cells by scFv and fusion

protein of se155-4 were the first records in the literature. Produced antibodies showed strong affinity to *S. typhimurium* LPS, live and killed *S. typhimurium*. With the recombinant production of se155-4 known as specific to serogroup B cells, its high affinity to Salmonella serogroup D LPS was indicated as the first, in this work. LPS was the best antigen with high affinity. $1.4x10^1$ *S. typhimurium* cells were the minimum pathogens detected by immunomagnetic ELISA.

Keywords: Gold nanoparticles, test strip, Salmonella, *E.coli*, scFv

ÖZ

GIDA PATOJENLERİ İÇİN İMMÜNOAFİNİTE TEMELLİ TEŞHİS PLATFORMLARININ GELİŞTİRİLMESİ

Çam, Dilek

Doktora, Biyoloji Bölümü

Tez Yöneticisi: Prof. Dr. Hüseyin Avni Öktem

Mart 2016, 164 sayfa

Gıda patojenlerinden Salmonella ve *E.coli*, sağlığı tehdit eden en önemli patojenler arasındadır. Bu patojenlerin hızlı ve kolay teşhisi büyük önem taşımaktadır. Bu kapsamda, çalışmanın ilk kısmında Salmonella ve *E.coli* O157:H7 için altın nanoparçacıklara (AuNP) dayalı, antikor temelli yatay akışlı test (YAT) platformu geliştirilmiştir. İkinci kısmında ise Salmonella'ya özgü se155-4 antikorunun tek zincirli değişken kısımları (scFv) ile SNAP-Tag içeren tam zincirli antikorları (füzyon protein) rekombinant olarak üretilmiştir. Bu antikorların hedef organizmayı yakalamaları normal ve immunomanyetik ELISA ile test edilmiştir.

Geliştirilen YAT platformunda HF180 en ideal membran seçilmiş, 36nm AuNP'lar ideal boyut olmuştur. En iyi tespit edilen türler *S. enteritidis* ve *S. infantis* olmuştur. *E.coli* hücreleri geliştirilen stripler ile spesifik şekilde yakalanmıştır. Stripler, hedef organizmaları bakteri karışımları içerisinde de hassasiyetle yakalamıştır. $5x10^5$ *S. typhimurium* ve $3x10^4$ *E.coli* bakterileri stripler ile teşhis edilen minimum hücre olmuştur.

scFv'ler hedef organizmayı tek başına $2.8x10^4$ hücre olarak yakalarken, farklı bakteri karışımı içerisinde $2.8x10^5$ hücre olarak yakalamıştır. scFv'lerin tam bir bakteri hücresini yakalaması ve Se155-4 antikorunun füzyon protein şekli ilk defa bu çalışmada geliştirilmiştir. Üretilen bu antikorlar, *S. typhimurium* LPS'lerine, ölü ve canlı *S. typhimurium* hücrelerine yüksek affinite göstermiştir. Serogrup B hücrelerine spesifik olduğu bilinen se155-4 proteinin rekombinant üretimi ile Salmonella serogrup D LPS'lerine de yüksek afinite gösterdiği ilk defa bu çalışmada belirtilmiştir. LPS, en iyi afinite gösterilen antijen olmuştur. İmmünomanyetik ELISA ile $1.4x10^1$ *S. typhimurium* hücresi teşhis edilen minimum patojen olmuştur.

Anahtar kelimeler: Altın nanoparçacıklar, test strip, Salmonella, *E.coli*, scFv

Dedicated to my lovely family, me and science

ACKNOWLEDGEMENTS

I would like to express my intimate and deepest appreciation to my supervisor Prof. Dr. Hüseyin Avni Öktem, who gave chance to me for improve myself in academic world everytime, for his encouragements, valuable advices, guidance and insight from beginning to end of this research. I would also like to thank Prof. Dr. Meral Yücel for her moral support, insight and continuous help throughout my graduate education. I have always been very pleasure to be in their research group.

I wish to express my intimate and deep gratitude to Prof. Dr. Andrew Ellington who affected my scientific point of view for giving me the opportunity to work with his research group and to learn new techniques at the Universtiy of Texas at Austin (UT). I wish to express my appreciation to Jimmy Gollihar for his continuous help in my research, kindness and good fellowship while I was at Ellington lab. I would also like to express my thanks to all members of Ellington lab, especially Arti Pothukuchy, Michelle Byrom, Andre Maranhao, Ross Thyer, Sanchita Bahadra, and Alison Moss for their kindly help at different parts of my work. I wish to thank to Prof. Dr. M. Stephen Trent and Dr. Shelley M. Payne for their kindly providing of bacteria needed for my research at UT.

I would like to thank to my thesis follow up committee members, Prof. Dr. Füsun Eyidoğan, Assoc. Prof. Dr. M. Cengiz Baloğlu and Assoc. Prof. Dr. Remziye Yılmaz for their critics and valuable suggestions.

I wish to thank all of the past and present members of the Plant Biotechnology and Nanobiotechnology R&D Research Group for creating a friendly and reliable atmosphere in the lab. I have always been very pleasure to be in lab and work with them. I would like to thank to Tahir Bayraç, Tufan Öz, Cengiz Baloğlu, Musa Kavas, Hamit Battal, Ayten Eroğlu, Ceren Bayraç, Oya Akça and Çağla Sönmez. I am also thankful to Emel, Şeyda, Ferhunde, Onur, Işkın, Ceren, Müslüm, Dilan and Melih.

I would also like to thank to members of Nanobiz Nanobiotechnological Systems R&D Limited, especially to Dr. Ceren Berkman and Özge Kalman for their friendly help at different parts of my research.

I wish also thank to TÜBİTAK (Scientific and Technological Research Council of Turkey) for financial support (2211 and 2214-A program) during my gradute education in Turkey and also during my research at UT.

As final, I would like to express of my thankful and all my love with appreciation to my lovely family. I am very thankful to my mom Hatice Çam, my dad Hayrullah Çam and my brothers Alper Çam and Akif Çam for their endless love, patience, encouragements and support. I feel always lucky for having them and their love in my heart.

This work was supported by the Faculty Development Program (ÖYP-BAP-08-11-DPT-2011K121010), and work related to conventional Lateral Flow Technology development and experimentation was supported by TÜBİTAK-TEYDEB projects funded to Nanobiz Nanobiotechnological Systems R&D Limited.

TABLE OF CONTENTS

LIST OF TABLES

TABLES

LIST OF FIGURES

FIGURES

LIST OF ABBREVIATIONS

Abs	Antibodies
BSA	Bovine serum albumin
BG	Benzylguanine
BHI	Brain-heart infusion
BME	β-mercaptoethanol
CSA	Common Structural Antigens
CDC	Centers for Disease Control and Prevention
CHO	Chinese hamster ovary
CDR	Complementary determining region
ELISA	Enzyme Linked Immunosorbent Assay
FT	Flow through
FK	Formalin killed
FPLC	Fast Performance Liquid Chromatography
GNPs	Gold nanoparticles
HEK	Human embryonic kidney
IPTG	Isopropyl β-D-1-thiogalactopyranoside
IMS	Immunomagnetic separation
IgG	Immunoglobulin G
IDT	Integrated DNA Technologies
Kan	Kanamycin
K_D	Dissociation constant
LOD	Limit of detection
LFIA	Lateral Flow Immunochromatographic Assay
LB	Luria Broth
LF	Lateral flow

LPS	Lipopolysaccharide
MAbs	Monoclonal Antibodies
Ni-NTA	Nickel-nitroloacetic acid
NC	Nitrocellulose
OD	Optical density
PBS	Phosphate buffer saline
PCR	Polymerase Chain Reaction
rxn	Reaction
RT	Room temperature
SPR	Surface Plasmon Resonance
scFv	Single chain variable fragment
TMB	3,3′,5,5′-Tetramethylbenzidine
TSB	Tryptic Soy Broth
TAE	Tris base, acetic acid and EDTA.
TEM	Transmission Electron Microscopy
VH	Heavy chain variable region
VL	Light chain variable region

CHAPTER 1

INTRODUCTION

1.1. Food Pathogens

Food safety is the most important issue for the public health throughout the world especially in developing countries. Because foodborne pathogens, e.g. viruses, bacteria and fungi cause not only serious damaging effects to food quality but also animal or human death (Mead et al., 1999, Oliver et al., 2005, Wallace et al., 2000). Infection of humans by those pathogens is occurred via contaminated sources such as drinking water and food (Dwivedi and Jaykus, 2011). Among 31 pathogens identified as causative agent for foodborne illnesses, Campylobacter, *L. monocytogenes*, *S. aureus*, Salmonella, *B. cereus*, *E.coli* O157:H7, *C. perfringens*, and *T. gondii* are the primary bacteria responsible for foodborne sickness, deaths and hospitalizations (Park et al., 2001, Gandhi and Chikindas, 2007, Murphy et al., 2006, Velusamy et al., 2010, Wang et al., 2011a). According to the estimates from US Centers, foodborne pathogens are in charge of 76 million cases of diseases which are occurred annually, and the annually cost caused by major pathogens containing *E.coli* and Salmonella was reported as about $ 6.9 billion (U.S. Food and Drug Administration, 2002, Allos et al., 2004). Besides, foodborne diseases were resulted by 3000 death and 128000 hospitalization according to the estimates of Centers for Disease Control and Prevention (CDC) in 2011 (CDC, 2011). Although foodborne illnesses might be increased via unhealthy nourishment, increased transportation and trade globalization (Käferstein et al., 1997), people have more awareness about foodborne pathogen contamination due to the increased facilities when compared to the past. In terms of the market, food sector has also remarkable potential for microbiological testing. Therefore, enhancing the quality of it or rapid detection of pathogens for food industry has become the priority in safety.

Thus, developing of cost effective, rapid, simple, disposable and easy to use assays without needed complex instrumentation with a high sensitivity is always the key issue in order to improve the food safety and public health.

1.1.1. Salmonella

Salmonella named from Salmon, American scientist discovered them, is widely distributed group of gram negative bacteria in nature and has about 2300 serotypes. The primary reservoirs of Salmonella are animals and humans. It invades the intestinal tract of hosts as a result of ingesting the contaminated foods and causes salmonellosis which might be resulted with death. *Salmonella enterica subsp. enterica* has almost 1500 serotypes which show various antigenic differences in H (flagellar) antigens and lipopolysaccharide (LPS) O antigens. Among the subspecies of *Salmonella enterica*; *typhimurium, typhi (choleraesuis), enteritidis, gallinarum, dublin and pullorum* are the major agents for human and animal diseases. According to research in 2004, 56% of the infections were accounted by *heidelberg*, 5%, *javiana*, 7%, *newport*, 10%, *enteritidis*, 15% and *typhimurium*, 20% (CDC, 2005). Generally, septicemia, enteric fevers and gastroenteritis are main three diseases caused by Salmonella species. Typhoid fevers are also serious problem occured by *S. typhi and S. paratyphi* infection while septicemia is caused by *S. choleraesuis*. In addition to this, *Salmonella* infections might be ended with a major economic losses in food industry (Levy, 1974). Besides, some species including *S. typhimurium* that is the most common causative agent for gastroenteritis (Aserkoff et al., 1970, Hargrett-Bean et al., 1988) can also be used as a bioterrorism agent via bacterial culture contaminated food and water supplies (Roffey et al., 2002). Most of the Salmonella infections which are transmitted to humans by poultry, pork, meat, chicken and eggs are typically food-borne illness. It was reported that around 15% of salmonellosis cases is caused by pork and 70% of pork contamination originates from Salmonella-infected pigs (Borch et al., 1996). Food borne salmonellosis may also be thought as caused by turkey products and meat as an important sources (Eurosurveillance Editorial Team, 2012).

In the United States (US), the outbreak of waterborne salmonellosis in California and Salmonella caused contaminated milk which gave rise to 16000 cases of human salmonellosis (Bean and Griffin, 1990) and 1.4 million cases of infection occured by Salmonella annually were reported (Olsen et al., 2001, Voetsch et al., 2004). Additionally, almost half of the gastrointestinal infections in the US alone each year is accounted for Salmonella contamination in food, and *S. typhimurium* seems to be responsible for many of gastroenteritis every year (Chalker and Blaser, 1988). In Europe, foodborne diseases caused by *S. typhimurium* became significant (Tsang et al., 1991) and *S. enteritidis* was the most common species which causes the 80% of food borned salmonellosis (Cogan and Humphrey, 2003). According to the data shown by the European Surveillance System 2010, 99.020 cases were caused by Salmonella through 27 European Union member countries and notification rate was reported as 21.5 cases/100.000 population (Eurosurveillance Editorial Team, 2012). Additionally, *S. typhimurium* or *S. enteritidis* were the cause of diarrheal patients in Korea (Kim, 2010). Salmonella species can also be a potential threat in Turkey since poultry and meat based nutrition is very popular. For instance, *S. corvallis* was reported as predominant seroytpe in collected samples such as poultry meat parts and ground turkey in Ankara (Hargrett-Bean et al., 1988). One of researchs also showed that 8.6% of total isolates from the wing part of chicken meat was *S. enteritidis* (Goncagül et al., 2005). Between the year of 1992 and 1993, *S. typhimurium* epidemics at the neonatology department (Ankara) was seen and resulted with 42 infected patients with meningitis, sepsis and gastroenteritis (Oral et al., 1995). According to the research in Diyarbakır, *S. enteritidis* caused foodborne outbreak in two private schools and four youth hostels and it was resulted with suffering of 346 people in 2009 (Dorman et al., 2011). İnce et al., reported that 1.38% of 29,601 diarrheal issues was caused by *S. enterica* and the percentage of serogroup D was higher than serogroup B (İnce et al., 2012). Figure 1 and 2 shows the distribution of gastroenteritis caused by Salmonella in Ankara/Turkey both yearly and monthly, respectively (İnce et al., 2012).

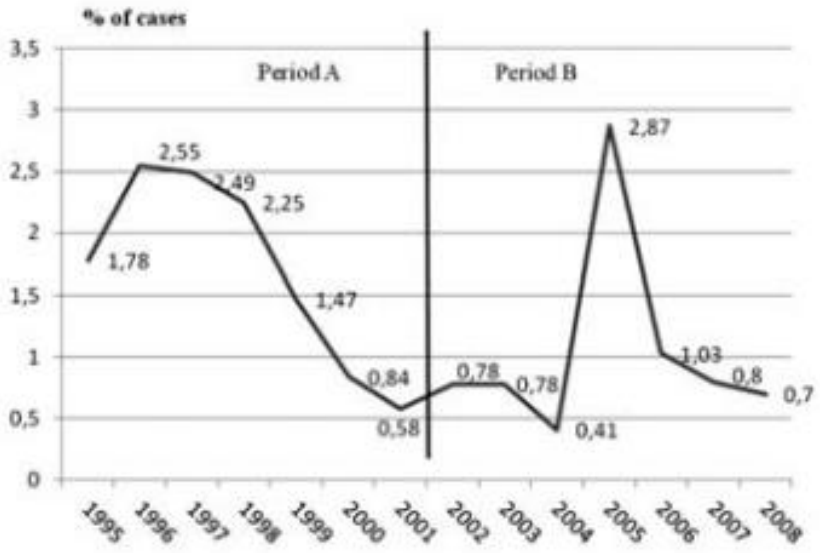


Figure 1. Distribution of gastroenteritis caused by Salmonella, yearly. (Taken from İnce et al., 2012)

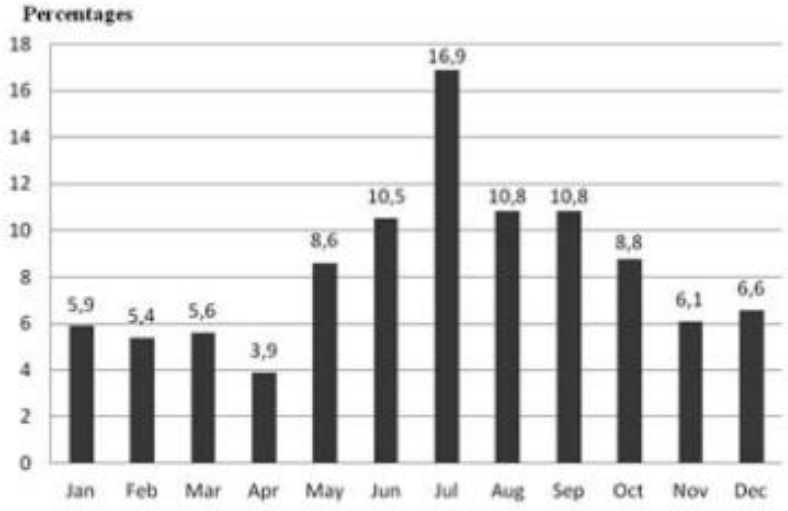


Figure 2. Distribution of patients by months. (Taken from İnce et al., 2012)

It is also possible that epidemiology of waterborne diseases might be occured by the global distribution of pathogenic bacteria via contaminated water movement (Altuğ, 2011) or some Salmonella carrier organisms in the sea. For instance, *S. typhi* was isolated in shellfish obtained from the contaminated region caused by slaughterhouse wastewater on the Aegean sea (Inal et al., 1979).

The outer membrane of Salmonella has three antigens including a) surface antigens, Vi antigens, which are occured in *S. paratyphi C, S. typhi,* and *S. dublin*. b) flagella antigens and c) somatic antigens, which are alcohol resistant and heat stable. The LPS structure is contained in somatic antigens and it may play a role as an endotoxin. LPS is also the major surface component of the outer membrane of gram negative bacteria. It is composed of i) an O-antigenic polysaccharides, which have protective role based on its length against the cationic peptides and bactericidal action of serum [Burns and Hull, 1998, Skurnik and Bengoechea, 2003), ii) an oligosaccharide core region and iii) the hydrophobic lipid A that anchors LPS to the outer membrane. Figure 3 shows the main LPS structure of *S. typhimurium* (Svenson and Lindberg, 1978, Hellerqvist et al., 1968).

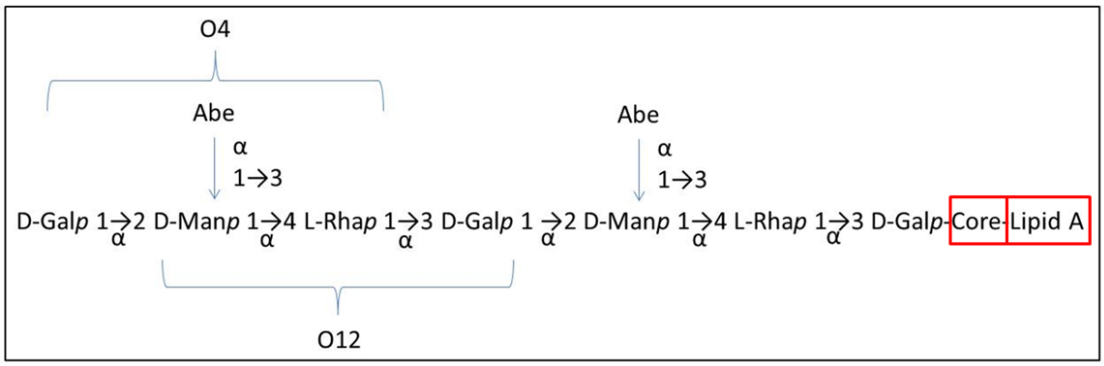


Figure 3. Main structure of octasaccharide or O polysaccharide chain of *S.typhimurium* LPS. OAc; O-Acetyl, Abe: Abequose, Gal: D-galactose, Rha: L-rhamnose, Man: D- mannose. (Adapted from Hellerqvist et al., 1968 and Svenson and Lindberg, 1978)

The differences of O antigens are resulted from the variations comes from the sugar components, their orders in the oligosaccharides and covalent bonds between the sugar molecules. As a result tri-tetra-penta or oligosaccharides form various repeating units in the O antigens. Salmonella O antigens are consisted of 10 variable monosaccharides and 5 common monosaccharides (Luderitz et al., 1966). In *S. typhimurium*, O antigen is composed of six to three sugar repeating units which extend out from the surface while fatty acids and sugars are the components of Lipid A (Nikaido, 2003, Raetz and Whitfield, 2002). Additionally, its O antigen is built from the four-sugar repeating unit

{→2)[αD-Abe (1→3)] αD - Man(1→4) αL-Rha(1→3) αD-Gal(1→}. In these antigens, an Abequose (3,6 dideoxyhexose) residue forms a branch point to the α-D-Man residue and antibodies (Abs) specific to three serogroups A, B, and D use the dideoxyhexose as the principal site for binding (Lüderitz et al., 1966). The demonstration of O4 and O12 antigenic repeats of *S. typhimurium* LPS is also shown at Figure 3. Many kinds of monoclonal Abs specific to LPS O antigens including O5 of B serogroup Salmonella are produced and tested by Enzyme Linked Immunosorbent Assay (ELISA) after reacting with *S. typhimurium* which has O1, O4, O5, and O12 antigens (Jaradat and Zawistowski, 1996).

A main-chain trisaccharide Mannose-Rhamnose-Galactose which is common to Salmonella serogroups A, B, D is the repeating unit of the Salmonella serogroup B-O antigen, but 3,6-dideoxyhexosyl group which binds to a Mannose in the repeating unit of the O polysaccharide by α 1→3 bond is the determinant group for those serogroups (Ekborg et al., 1977). It is known that the O antigens from *S. enteritidis* (serogroup D) (O:1,9,12) are similar to O antigens of *S. typhi* (serogroup D) (O:9,12), *S. paratyphi B* (serogroup B) (O:1,4,5,12) and *S. typhimurium* (serogroup B) (O:1,4,5,12). Table 1 indicates the O polysaccharide structures of some Salmonella serogroups (C, D, O66) which were also used in this study.

Table 1. Structures of Salmonella O polysaccharides. Man: Mannose, Rha: Rhamnose, Gal:Galactose, Tyv: Tyvelose, Glc: Glucose, GlcNAc: N-acetyl-D-glucosamine, Abe: Abequose, GalNAc: N-Acetylgalactosamine

O9 (D_1) *S. typhi*	2) Man (α1-4) LRha (α1-3) Gal (α1- | | Tyv (α1-3) Glc2Ac (α1-4)	(Jann and Westphal, 1975) (Rahman et al., 1997) (Brooks et al., 2008)
O6,7 (C_1) *S. ohio*	2) Man (β1-2) Man (α1-2) Man (α1-2) Man (β1-3) GlcNAc (β1- | Glc (α1-3)	(Di Fabio et al., 1989)
O8 (C_2) *S. newport*	4) LRha2Ac (β1-2) Man (α1-2) Man (α1-3) Gal (β1- | | Abe (α1-3) Glc2Ac (α1-3)	(Wilkinson, 1977) (Hellerqvist et al., 1972)
O66	2) Gal (α1-6) Gal (α1-4) GalNAc (α1-3) GalNAc6Ac (β1- | Glc (β1-3)	(Liu et al., 2010)

1.1.2. *E.coli*

E.coli bacteria which have many types of strains live in the gastrointestinal tract of humans and animals. Although lots of them are harmless some strains including *E.coli* O157:H7 can cause death. After outbreaks seen in the year of 1982, *E.coli* O157 was reported as a food pathogen (Riley et al., 1983). *E.coli* O157:H7 is known as the significant agent that leads to the haemorrhagic colitis and one of the most important foodborne pathogens transmitted from animals to humans (Altekruse et al., 1997, Slutsker et al., 1998). Besides, food and waterborne diseases are mainly caused by this strain. Transmission of this pathogen to the humans might be occurred by contacting with animals, eating contaminated foods especially ground beef or drinking contaminated water, and passing one person to another directly (Heiman et al., 2015). To highlight, the major reservoir of this pathogen is cattle (Dorn and Angrick, 1991, Slutsker et al., 1998, Wells et al., 1998). Infections of humans with *E.coli* O157:H7 can be resulted with clinical issues containing haemolytic uremic syndrome, acute nonbloody diarrhea and thrombocytopenic thrombotic purpura. Furthermore, killing of intestinal cells, destruction of kidneys, paralyses or respiratory defects can be occurred by a few *E. coli* O157:H7 particles such as in a number of ten to a hundred (U.S. Food & Drug Administration, 2002). According to the outbreaks caused by *E.coli* O157:H7 which was seen at between 2003 and 2012, it was more compared to the previous years in the US (Heiman et al., 2015). Besides, hospitalizations and infections caused by *E.coli* O157 are still being continued according to the food safety news.

Since pathogenic *E.coli* can contaminate the food and water easily (Liu and Li, 2002) and it causes severe defects in humans and animals, the sensitive and rapid detection of this bacteria has always become crucial for public health. There are numerous methods including Ab based microarrays (Gehring et al., 2006). However, traditional cultural technique involving the identification of bacteria according to their characteristics morphologically, biochemically and immunologically remains as routine method for detection even if it is time consuming and difficult to interpret the results (Sheridan et al., 1998).

1.2. Methods in Pathogen Detection

To ensure the public health, early detection of pathogens contaminated the foods or consumption products is crucial issue especially for the government authorities. Therefore, many kinds of identification methods or microbiological tests are in use and new detection platforms are also being tried to develop for improving the sensitivity, selectivity of detection with a low cost and rapid tests.

1.2.1. Standard culture methods

Traditionally, the Salmonella diagnosis in the laboratory is based on selective enrichment and culture which is performed subsequently on selective medium (Stone et al., 1995) and then biochemical and serological confirmation tests are needed. Briefly, after growing of sample in the culture media for selection of *Salmonella*, it is needed to be isolated. After that ELISAs and enzyme immunoassays (EIAs) that are a kind of serological and biochemical tests are used in order to verify the presence of *Salmonella* (Waldmann, 1991, Plückthun, 1991). Although the detection of bacterial species and also of Salmonella in foods by conventional bacteriological culture method seems to a gold standard for sensitive detection (Andrews and Hammack, 2001), these steps require 5 or 7 days which is incovenient for food sector or industrial applications.

1.2.2. Molecular methods

Polymerase Chain Reaction (PCR) is commonly used technique in order to detect the microbes based on specific nucleic acid amplification. In some cases it has a potential to replace traditional methods for bacterial characterization and identification in foods. It has also been a popular approach compared to biochemical characterization for Salmonella, but conventional PCR techniques may suffer from having low sensitivity of accumulated DNA samples and only qualitative results can be provided by it. With the developing of new instrumentation and chemistries, real time observing of nucleic acid amplification continously is possible with real time PCR which enables to detect the Salmonella (Malorny et al., 2004) and *E.coli* O157:H7 (Fu et al., 2005). Time saving is the main advantage of real time PCR, and the detection of Salmonella is

possible with the commercially available real time PCR based assays. Multiplex PCR, another molecular method, is based on several primer sets and their combination into single PCR. It represents a rapid and reliable method to identify of lots of numbers of serotypes compared to the methods based on phenotypic serotyping. There are numerous studies that show the ability of the detection of different serogroups of Salmonella and *E.coli* O157:H7 by multiplex PCR (Wang et al., 2007) and real time multiplex PCR in chicken samples or other sources (Woods et al., 2008, Kim et al., 2006, O'Regan et al., 2008, Rajtak et al., 2011).

Reverse transcriptase PCR (RT-PCR) is also used for pathogen detection. The enzyme reverse transcriptase is used for reverse transcription of RNA into its complementary DNA (cDNA) in RT-PCR, and then cDNA is amplified by conventional PCR techniques. Although this method might improve the sensitivity, it has some limitations such as labor intensive, rapid extraction of RNA, cross contamination and DNase treatment. Although there are a few studies were reported for Salmonella and *E.coli* O157:H7 detection by RT-PCR technique (D'Souza et al., 2009, Park et al., 2011, Yaron and Matthews, 2002), its application to the bacterial detection in the environmental samples or food is limited. Therefore, reliable extraction procedures or recovery are still in need.

Nucleic acid or DNA hybridization assay based on the probes directed to specific nucleic acid regions of microbes might be used for the detection as a powerful tool, and commercial hybridization assays are also available (D'Aoust et al., 1995). This technique offers more sensitivity and specifity compared to the agarose gel electrophoresis or standard culture based methods (Manzano et al., 1998) and eliminates the cross reactivity with non target organisms and serological confirmation. For instance, PCR products of *S. typhimurium* was detected by hybridization technique (Maciorowski et al., 1998) and it can also allow targeting of multiple genes of Salmonella samples by DNA microarray technology.

Phage based detection methods are also included in molecular methods. Bacteriophages are host-specific viruses that infect bacteria and have DNA or RNA genome encapsulated by proteins. Since they lack their own metabolism, they inject

their genome into the bacterial cytoplasm and then replicate**.** Therefore, virulent phages within the bacteria can be adapted for detection purpose since they cause the death of their host because of unabling to integrate into the genome Hagens and Loessner, 2007). It is known that Vidas Up Salmonella solution (Biomerieux) containing phage recombinant protein obtained from bacteriophage fibers is used for the detection of Salmonella in food within 18-24 hours.

1.2.3. Immunological based methods

Mass-Based immunosensors, thermometric and magnetic immunosensors, optical immunosensors are available techniques for the detection of bacteria but they are not in common usage. Immunomagnetic separation (IMS) is the most succesful approaches for the capturing or isolation of target organism rapidly and specifically (Shaw et al., 1998). Magnetic capture is based on magnetic bead from nanometer scale up to micrometer conjugated with Ab or oligonucleotide that will capture the agents and then magnetic separation via magnetic force, simply. It was reported that IMS is able to reduce the time needed for total culture analysis and it is more sensitive than common culture methods (Lynch et al., 2004). Disadvantage of this strategy is that it might cause target loss. IMS can be coupled with other methods including conductance microbiology, ELISA, electrochemical detection (Perez et al., 1998), PCR (Liébana et al., 2009), and fluorescence observation (Zhao et al., 2009, Yang and Li, 2006) in order to increase the analytical sensitivity (Fluit et al., 1993, Cudjoe et al., 1994, Cudjoe et al., 1995, Spanová et al., 2000). For instance, *S. typhimurium* was recognized by immunomagnetic nanospheres and immuno fluorescent nanospheres with 10 cfu/ml detection limit (Wen et al., 2013). It was also reported that very low cell numbers of *E.coli* O157:H7 was detected in aqueous sample by combined immunoliposome-immunomagnetic bead based assay (DeCory et al., 2005). Although some kind of IMS beads for recovering the Salmonella from foods are available in commercially, Dynabeads^{TM} anti Salmonella (Invitrogen) is found to be the most common. Magnetic beads like Captivate Salmonella (Lab M) and Tecra Salmonella Unique (3M) specific to Salmonella and Rapidchek Confirm *S. enteritidis* IMS kit (SDIX) are the other commercially available detection tools.

ELISA technique is used to detect the presence of an antigen or Ab in a sample. It is easily adaptable to the technology used for microbiological testing and it is also highly sensitive, spesific and easy to perform. The limitations of common ELISA are the high detection limit of bacteria, long testing time and cross reactivity. However, various kinds of ELISA based detection methods including gold nanoparticles (GNPs) (Ambrosi and Merkoçi, 2009) are being in develeped for further inceasing of sensitivity and decreasing the detection limit. In 1977, the first Salmonella detection assay by enzyme immunoassay was reported (Krysinski and Heimsch, 1977). Currently Salmonella detection can be perfomed by ELISA plate based assays such as TRANSIA®PLATE Salmonella Gold (BioControl), RIDASCREEN® Salmonella ELISA (R-Biopharm AG) and Salmonella ELISA (BIO ART SA). Different types of LPS and Lipid A (Freudenberg et al., 1989) or bacterial cells can be used for coating the ELISA plate as an antigen. PBS, carbonate buffer in a different molarity can also be used for LPS or live&killed bacterial cell coating (Siragusa and Johnson, 1990, Wu et al., 2014) but Covalink plates are recommended for rough LPS coating [105]. The detection of *E. coli* O157:H7 comes from animal and human samples by ELISA is also commonly used technique (Kerr et al., 2001).

Rapid agglutination assays are other immunological based methods. Several agglutination assays based on latex particles are in use for the identification of different Salmonella serogroups as commercial kit (OXOID Salmonella latex test, Microgen® Salmonella rapid test). However, these type assays are used as confirmative tests after selecting the Salmonella colonies on the selective agar plate for further identification. Test might be resulted with non-specific agglutination with some other organisms.

Many of the mentioned techniques above require skilled personel, sterile working conditions, sensitive and costly equipment in order to perform sensitive and specific microbial analysis and also most of these systems can not be used in field (Sapparapu, 2003) and they are not portable. However, in order to develop the sensitive and fast method for the bacterial antigens, many studies have been done to enhance each of these steps (Le Gall et al., 1998, Michael et al., 1996). For instance, MicroSnap-*E.coli* device which can be supplied by Hygiena was used for rapid detection of *E.coli* (http://www.hygiena.com/microsnap-e-coli-other.html).

1.2.4. Biosensors

The combination of chemistry, biology, engineering and other multidisciplinary researches has created a biosensor technology which is classified based on the transduction devices and biorecognition principles. The first example of developed biosensor was for the detection of glucose level in serum and based on using immobilized enzyme (Clark and Lyons, 1962). The sensing of electrochemically active metabolites or respiration activity is also adapted for microbial biosensors (Yu et al., 2006). On the basis of the biorecognition elements, biosensors can be categorized as bioaffinity and biocatalytic sensors. Besides, optical, thermal and magnetic sensors, piezoelectric and electrochemical (Tothill and Turner, 2003) biosensors are classifed according to the transducing element.

Electrochemical biosensors have biorecognition element which can be an Ab, nucleic acid, enzyme, cell (Kerman et al., 2004, Wang, 1999, Wang, 1998, Wang et al., 2011b) and transducer to convert the recognition into a measurable equivalent electrical signal. Amperometric, potentiometric, impedimetric, conductiometric biosensors can be classified in electrochemical biosensors. However, immobilization of biological molecules on electrode substrates is the major challenge of this type of sensors. *S. typhi* (Rao et al., 2005), *E.coli* O157:H7 (Lin et al., 2008), *E.coli* (Arora et al., 2007, Shiraishi et al., 2007), Salmonella species (Ma et al., 2014) including *S. enteritidis* (Zhang and Alocilja, 2008) and *E.coli* O157 were detected by either electrochemical biosensors or label free electrochemical immunosensor (Yang et al., 2004).

Optical biosensors are another type of bioensors and they are desirable for label free detection of bacteria (Peng and Miller, 2011) such as *E.coli* (Baeumner et al., 2003, Rahman et al., 2006) *Salmonella enterica* (Zuo et al., 2013) and *S. typhimurium* (Seo et al., 1999). Surface Plasmon Resonance (SPR), Raman&FTIR, Fiber optic sensors are included in this type of sensors (Velusamy et al., 2010). SPR is the common base to detect the foodborne pathogens using reflectance spectroscopy. *E.coli* O157:H7 and *S. typhimurium* with a 10^2 cfu/ml (Waswa et al., 2007) and $1x10^6$ cfu/ml (Laura et al., 2011) detection limit, respectively were detected by SPR biosensor (Oh et al., 2005).

Microfluidic biosensors are also becoming attractive for improving the performance of bacteria biosensors (Mernier et al., 2010, Boehm et al., 2007, Bayoudh et al., 2008, Liu et al., 2007). Polydimethylsiloxane substrate is used for fabrication of the network of microchannels and superparamagnetic beads are used as a mobile solid support for developing microfluidics systems. 1-100 mm range is used as the critical operational length for microfluidics while 1–100 nm is for nanofluidics (Prakash et al., 2009, Yanik et al., 2010). Positive pressure gradient is used for the flow of fluidics and the flow of solution continuously is commonly performed up to 80 µl/min. The principle of biosensor is based on the hybridization of DNA/RNA and signal amplification using liposome. Shorting the analysis time up to 15 min (Zaytseva et al., 2005, Lui et al., 2009, Mairhofer et al., 2009), reducing of reagents needed, increasing sensitivity and reliability in a single device are some advantages of microfluidics. Developing the disposable (Gottschamel et al., 2009) and low cost microfluidics are also new trends for improving this system. It has been reported that *E. coli* O157:H7 was detected using microfluidics systems as a detection limit of $1.2x10^3$ and $1.6x10^2$ cfu/ml in ground beef sample and pure culture, respectively (Varshney, et al., 2007), and *S. typhimurium* microfluidic nanobiosensor (Kim et al., 2015) was also developed.

Although Ab and nucleic acid based sensors (Velusamy et al., 2010) are commonly used for foodborne pathogens detection, lectin and bacteriophage-based sensors can also be used. Lectin is a kind of glycoprotein and it can bind to the components of polysaccharide structures of bacterial cell surfaces. For instance, *E.coli* was reported with a label free detection by using lectin modified electrodes (Gamella et al., 2009). It was also shown that bacteriophage based biosensor was developed for *E.coli* by Gervais et al. (Gervais et al, 2007).

S. typhimurium detection by wireless magnetoelastic (ME) biosensors (Chai et al., 2012), nanobiosensor (Kim et al., 2013) and the recognition of *S. enteritidis* by GNP-DNA biosensor (Alocilja et al., 2013) was also reported in the literature. *E. coli* O157:H7 recognition by piezoelectric biosensors (Wu et al., 2007, Su and Li, 2004), and the detection of *E. coli* O157:H7 and *S. typhi* with a $4.12x10^2$ cfu/ml (Li et al.,

2012) and 10^5 cells/ml (Singh et al., 2005), respectively by immunosensors are another examples of biosensor based pathogen detection.

To say that biosensor based microarray technology is also offering advantages for sensitive microbial detection. Since the pathogen detection using biosensors is new concept, it has limitations such as the formation of sensor surfaces, sample preparation, renewability and reproducibility. To commercialize the biosensors, some requirements are also needed. They are specifity, sensitivity, stability, reproducibility, response linearity, short recovery and response time of biosensor devices (Thakur and Ragavan, 2013). However, biosensors for pathogen detection seem to have potential to be a more powerful with the combination of new trend technologies involving nanotechnology as they can minimize the immobilization problem of molecules to obtain proper signal.

Apart from the biosensors, future trends such as cell phone based sensing (Zhu et al., 2012) are still in need. Because very low cell number, <100 cfu/g, (Hoorfar, 2011) of foodborne pathogens is present in the samples and it is hard to detect them among the other bacteria. Real time testing is one of the trends planning to be used in foodborne pathogens detection. If it is succeded to generate results within near real time, online monitoring or on-farm monitoring could be possible. Because being a mobile, virtual, digital and personal of diagnostic tools is crucial for diagnostic technologies. It can also enable to usage of them at the source of infection such as animal, field, etc. Metagenomics, culture-independent detection and typing might also be included in the new trends of pathogens detection (Hoorfar, 2011).

1.3. Antibodies for diagnosis

1.3.1. Antibody production

In the development of new drugs and immunoassays for the treatment of human diseases or bacterial detection, Abs are valuable reagents because of having high specificity for their antigens. Hybridoma technology was developed by Milstein and coworkers in the early 1970s, and prepared for the first time monoclonal Abs (MAbs) which have been widely used in therapeutics and clinical diagnostics (Kricka, 1998)

of predetermined specificity (Köhler and Milstein, 1975). The generation of proteins is essential in appropriate quality and quantity at the present time. Therefore, engineering Abs with desired characteristics have become popular by the advancement in recombinant DNA technology, recently. For that purpose, the application of bacterial cells and mammalian cells for producing proteins is being progressed. Common structure of full length immunoglobulin G (IgG) Ab is shown in Figure 4.

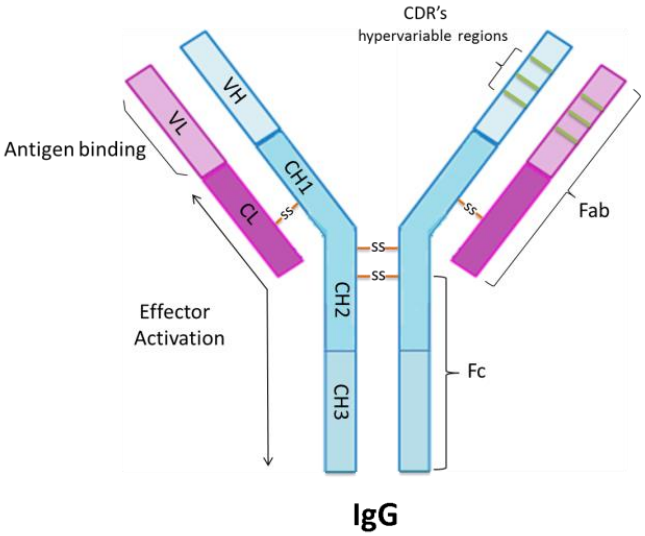


Figure 4. Schematic representation of typical full length IgG Ab. CDR: Complementarity determining region, Fab: Fragment, antigen binding, Fc: Fragment, crystallizable, VH: Variable domain, heavy chain, VL: Variable domain, light chain, CL: Constant domain, light chain, CH: Constant domain, heavy chain

Expression system in *E.coli* is very common in order to produce the recombinant Abs, single chain variable fragments (scFvs) and Fab molecules while various bacteria are used as host (Sodoyer, 2004). *E.coli* is known as the basis for endeavours in protein engineering (Gordon et al., 2008) since it has efficient transformation capability, low cost fermentation possibility, greater ease of genetic manipulation and availability of many mutant host strains and cloning vectors. BL21 and K12 are the most frequently used *E.coli* strains for routine expression of protein (Terpe, 2006). Generally, overproduced recombinant proteins are accumulated into the periplasmic space or

cytoplasm. Although *E.coli* periplasm offers correct folding and processing to Ab chains, inclusion bodies known as insoluble disulfide proteins in cytoplasm come from overproduced proteins might be occurred. The formation of inclusion bodies can make easy to purify the protein but it is not guaranteed that refolding will give a biologically active yield. Because misfolded protein and affinity tags may cause decreased activity of product.

Mammalian cells are also currently used hosts to generate the commercial proteins such as monoclonal Abs (Mohan et al., 2008, Melton et al., 2001, Chu and Robinson, 2001, Andersen and Reilly, 2004). The expression of proteins in those cells may also be achieved by viral mediated transduction (Boyce and Bucher, 1996). Chinese hamster ovary (CHO) cells, mouse myeloma cells and HEK 293 cells are the most widely used host cells (Griffin et al., 2007). Calcium phosphate or polyethyleneimine can be used for transfecting those cells (Huh et al., 2007). Post translational modifications, product assembly and pertinent protein folding required for biological activity can be introduced by this espression system. Another advantage of it is that mammalian cells recognize the signals necessary for synthesis and secretion of proteins, efficiently (Khan, 2013). However, high cost, complicated technology of system and contamination risk are the some limitations of mammalian cell based expression (Yin et al., 2007), but it can be improved by using properly designed vector (Jalah et el., 2007).

There are numerous proteins produced against to Salmonella, *E.coli* and other bacteria using hybridoma or recombinant technologies (Köhler and Milstein, 1975, Siegel, 2002). Many of produced Abs are specific to LPS structure of bacteria but some Abs were developed against outer membrane proteins (OMPs) of bacteria including *S. typhimurium* (Zhang et al., 2006a). Although some of them react with LPS O4 antigens specifically, others cross reacted with serogroup A (Tsang et al., 1991).

1.3.2. Se155-4 antibody specific to Salmonella

Se155-4 is IgG1/ʎ1 mouse hybridoma Ab and it is known as specific to Salmonella serogroup B in the literature. It offers several advantages as a model system for

producing Ab. These include well clarified crystal structure (Cygler et al., 1991), a detailed description of binding thermodynamics (Sigurskjold et al., 1991, Sigurskjold and Bundle, 1992), and an efficient *E.coli* expression system for Fab (Anand et al., 1991a) and scFv (Anand et al., 1991b). In comparison to other carbohydrate binding proteins, its binding site is unusual. For instance, lipophilic interactions, Van der Waal's and hydrogen bonding are provided merely by a phenylalanine, two histidines and three tryptophans that line the base of the binding pocket (Cygler et al., 1991). The Ab binding sites of se155-4 are constructed from six segments, three from each chain, termed as CDRs (complementary determining region). It is generally known that aromatic amino acids are included in the CDR of Ab in a high frequency (Padlan, 1990). se155-4 Ab binding site has this characteristic and Abequose of the trisaccharide epitope of LPS structure is buried in it, while the galactose and mannose residues are partly solvent exposed and also both of them make hydrogen bond with Ab (Zhang and Bundle, 2000, Cygler et al., 1991, Bundle et al., 1994a, Zdanov et al., 1994). It also shows pH dependence of antigen binding (Bundle et al., 1994b) attributable to a fifth His situated in the antigen-binding pocket. The structures of ligands for the Se155-4 Ab are shown in Figure 5 (A, B). The studies related with protein engineering of Fab and scFv of se155-4 implicated that this Ab has a notable tolerance for mutations which close to the binding pocket (Brummell et al., 1993, Deng et al., 1994, Deng et al., 1995). For instance, the modification of se155-4 Ab's Cys94L in scFv version had a slightly reduced binding profile to its antigen (Young et al., 2014).

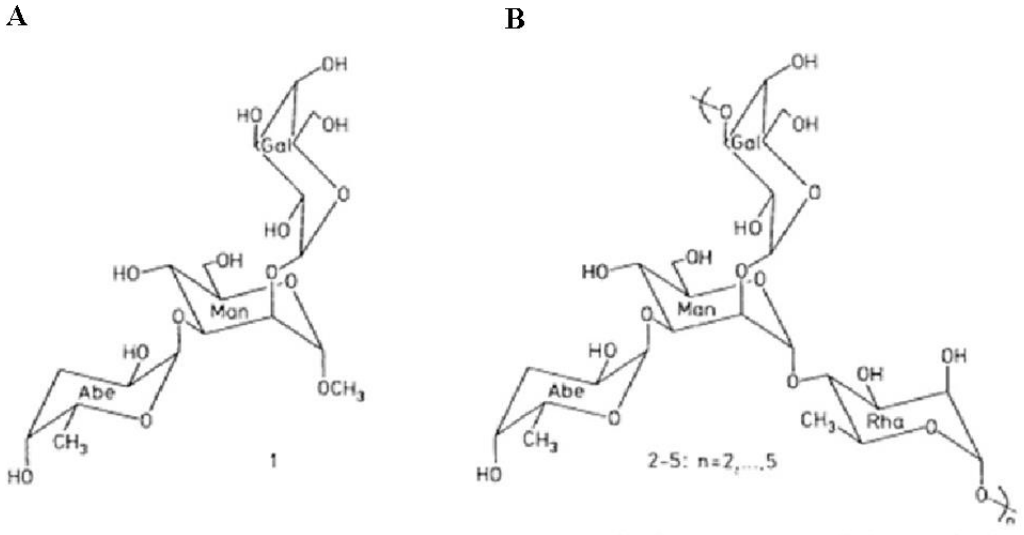


Figure 5. Schematic representation of structures of the ligands for the antibody Se155-4 **(A, B)**. Methyl glycoside of Salmonella serogroup B 0-antigenic trisaccharide epitope bound by antibody Sel55-4 **(A)**, Tetrasaccharide repeating unit of the 0-antigen **(B)**. The glycosidic linkages are (→3)-α-D-Gal*p*(l→2)-[α-D-Abe*p*(l→3)]-α-D-Man*p*(l→4)-α-L-Rha*p*(1→). Abe: Abequose (3,6-dideoxy-xylo-hexose), Rha: Rhamnose (6-deoxymannose). (Taken from Sigurskjold et al., 1991)

Van der Waals interaction and Hydrogen-bond contacts between the Fab fragment and oligosaccharides was also given in Table 2 and Table 3, respectively (Cygler et al., 1991).

Table 2. Van der Waals contacts between the Fab fragment and oligosaccharide. Numbers in parentheses: Atom-atom contacts <4.2 A˚. (Taken from Cygler et al., 1991)

Sugar	Fab residues or ordered water
Gal	His 32L1 (7), Trp 91L3 (15), Asn 94L3 (2), His 97H3 (1)
Man	Trp 33H1 (3), is 97H3 (7), Ser 202H* (2)
Abe	Trp 91L3 (9) , Trp 96L3 (6), Trp 33H1 (13), His 35H1 (4), Phe 58H2 (2), Gly 96H3 (4), His 97H3 (4), Gly 98H3 (5), Tyr 99H3 (1) Wat 1 (7)

Table 3. Hydrogen-bond connections between the oligosaccharide and Fab fragment and ordered water. (Taken from Cygler et al., 1991)

Antigen atom	Contacting atom	Distance (A^o)
Gal, O-2	0-1, Abe	3.00
Gal, O-2	0-2, Abe	2.68
Gal, O-3	Nϵ2, His 32L1	3.01
Gal, O-4	Nϵl, Trp 91L3	3.04
Man, O-4	Nδ1, His 97H3	3.21
Abe, O-2	N, Gly 98H3	2.68
Abe, O-4	Nϵl, Trp 96L3	2.86
Abe, O-4	O, Wat 1	2.84
Abe, O-5	O, Wat 1	3.11

In terms of the binding constant, the affinity of se155-4 anti Salmonella Ab to BSA-O-polysaccharide conjugate was reported as $2x10^5$ M^{-1} (Brummell et al., 1993). Another study also demonstrated that the dissociation constant (K_D) value of Fab is similar to scFv monomer which is $6.5x10^{-6}$ M while it is $1.4x10^{-7}$ M for se155-4 IgG and $7.2x10^{-8}$ M for scFv dimer (MacKenzie et al., 1996). Additionally, 10 μM of scFv (Young et al., 2014), 7.5 μM of IgG (MacKenzie et al., 1996), $8x10^{-8}$ M^{-1} of dimeric form Ab and $4x10^{-6}$ M^{-1} of the monomer Ab (Svenson and Lindberg, 1978) were among the reported K_D values of different forms of se155-4 Ab.

Some kinds of Abs were produced against to different O antigenic structures of Salmonella. To give an example, monoclonal Abs specific to LPS O antigen of B serogroup Salmonella were generated. Their binding was tested with commercially available LPS from *S. typhimurium* and IgG MAbs exhibited lower avidity than the IgM (Jaradat and Zawistowski, 1996). Deng et al., has developed the scFv of Se155-4 with some mutations by chemical and error prone PCR methods and it was reported that only the variable heavy chain (V_H) libraries yielded clones with improved binding to serogroup B LPS (Deng et al., 1994). Wang et al., (2001) developed fusion protein between an anti Salmonella scFv and the photo-protein aequorin (scFvLAEQ). They demonstrated that it maintained the Ab binding affinity toward the Salmonella antigen

and the detection limit of antigen was 10 µg/ml. Anand et al., (1991b) investigated the effects of peptide linker design and variable light chain (VL)-VH orientation on expression, secretion, and binding to a Salmonella 0 polysaccharide antigen. According to the results, main order and linker sequence affected antigen binding and the thermodynamic binding parameters of intact and cleaved scFvs were similar to those of bacterially produced Fab and mouse IgG. scFv of se155-4 Ab was also generated by modifying the free Cystein residue in parental Ab and the binding affinity of it was ~10 µM (Young et al., 2014). Morever, it was shown that the bacterially produced Fab of se155-4 was as active as purified mouse Fab (Anand et al., 1991a). Selected scFvs for *Salmonella enterica* from an immunized scFv library was also used for the detection of this pathogen (Cannon and Heinhorst, 2010).

1.3.3. Single Chain Variable fragments (scFv)

scFv (Wannlund et al., 1980, Matveev et al., 1999, Brichta et al., 2003, Dolezal et al., 2003, Knight, et al., 2008) that comprises of a single polypeptide chain with similar antigen binding affinity as its parent Ab is an example of attractive recombinant protein technology. The creation of scFv is based on the linking the variable domains of the VL and VH through a flexible linker polypeptide region which is generally (GGGS)x3. The hydrophilicity of the peptide backbone is enhanced by glycine and serine residues included in linkers and they provide flexibility of protein (Dolezal et al., 2003). The length of the linker affects the molecular association of produced scFv. For instance, while the length is longer than 12 residues, monomeric scFv is occured (Hudson and Kortt, 1999, Kortt et al., 1994) and shorter linkers enhance the occurence of multimeric or dimeric scFvs (Plückthun and Pack, 1997, Atwell et al., 1999). However, when the linker length is under three residues it improves the assembly of scFvs into triabodies or trimers (Hudson and Kortt, 1999, Atwell et al., 1999). It is worth noting that the solubility and stability of expressed scFv are affected by the length and amino acid sequences or composition included in peptide linker (Robinson and Sauer, 1998). As a flexible linker $(G_4S)_3$ has been widely used as common polypeptide linker. Karsunke et al., (2012) reported that VH has major role for binding to antigen and VL might increase the final sensitivity for target or it may provide cross reactivity.

A variety of scFvs are desirable for in vivo diagnostic and wide medical applications in clinical immunoassays [Luo et al., 1998, Reiter and Pastan, 1998) in vitro imaging (Lizano et al., 1997), signaling (Yang and Li, 2006), immunotoxin therapy (Nedelman et al., 1993), radio-immunotherapy (Yokota et al., 1992, Nieroda et al., 1995) and chip technology (Wang et al., 2006). In addition to this, scFvs have potential advantages compared to whole Abs. a) They are resulted in faster clearance from the body&tissue (Colcher et al., 1990) when they are intravenously administered. Because they are about one fifth the size of full length IgG Abs (Holliger and Hudson, 2005) with a molecular weight of about ca. 30 kDa. b) They can retain full antigen binding capacity. c) scFv Abs do not contain the Fc region, therefore they are not able to trigger potentially harmful immune responses (Holliger and Hudson, 2005) unlike whole Abs. d) scFvs are available in recombinant form and they can be generated in non-mammalian systems (Tolner et al., 2006a, Tolner et al., 2006b) with site-specific tags for purification. Although it is known that the binding affinity of scFv has almost the same with parental full length IgG Abs (Pleckaityte et al., 2011), Wels et al., (1992) showed that the binding affinity of original monoclonal Ab is 9 fold higher than of produced scFv to whole cell. There are lots of poduced scFv fragments such as anti 2,4-dichlorophenoxyacetic acid (2,4-D) herbicide hapten (Brichta et al., 2003), scFv which recognizes Cry1C in food samples (Wang et al., 2012), anti-MUC1 scFv for tumor cells (Wu et al., 2014) and scFv specific to complement receptor 1 in *S. gordonii* (Knight et al., 2008). scFvs can also be used for developing lateral flow immunochromatographic assay (LFIA) using GNPs as conjugate, but it may be failed to detect the target like *M. tuberculosis* cell extracts (Fuchs et al., 2014).

Typically, the generation of scFv is based on small scale expression but it can also be performed by large-scale production (Miethe et al., 2013). While the *E.coli* expression is very common for the production of recombinant scFv proteins, mammalian cells (Jäger et al., 2013), wheat germ (Kawasaki et al., 2003) and transgenic tobacco plants (Makvandi-Nejad et al., 2005) were also reported. Cloning of scFv genes or recombinant proteins is commonly achieved by pET system since it is the most powerful sytem for expression of recombinant proteins using *E.coli* bacteria. Briefly,

bacteriophage T7 controls the cloning of target genes in pET plasmids, expression is achieved by T7 RNA polymerase (pET System Manual, Novagen).

1.3.4. Fusion proteins and SNAP-Tag technology

Fusion proteins are becoming popular interest for developing new and functional proteins for imaging and detection technologies. It basically depends on attaching the functional tags to the C or N terminals of proteins. The SNAP-Tag is produced via engineering of AGT (O(6)-alkylguanine DNA alkyltransferase) which is included in the human DNA repair enzymes. It is known that this version of AGT has 52 fold increased activity towards BG (benzylguanine) substrate (Gronemeyer et al., 2006). The SNAP-Tag is used for producing fusion proteins and it seems that it is an ideal tool for specific protein labelling. SNAP-Tag labelled proteins or Abs can be used for detection of targets or antigens via magnetic beads which have the BG substrates (Figure 6). Green florescence protein (GFP) fused scFv fusion protein was reported and it was shown that fusion partners did not influence the behaviours of each others, and the binding affinity of fusion Ab was almost identical with scFv alone (Hink et al., 2000). Photoprotein aequorin (AEQ) and scFv of se155-4 Ab was also fused [Wang et al., 2001) and both binding affinity of scFv domain and bioluminescence properties of AEQ were maintained.

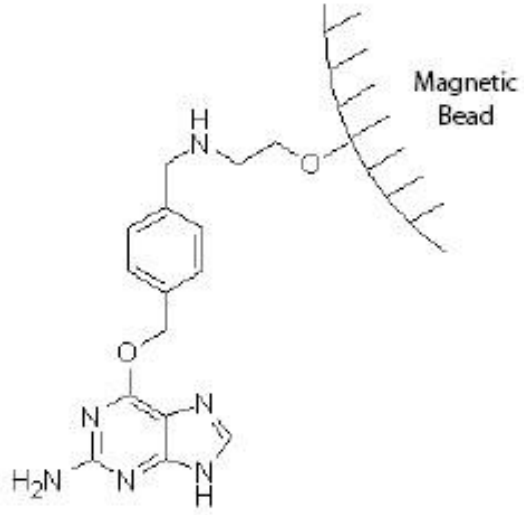


Figure 6. The coupling of SNAP-Tag substrate BG with highly stable 75-150 μm superparamagnetic particles (NEB)

1.4. Nickel Affinity Chromatography (Ni-NTA)

Ni-NTA is a type of Immobilized Metal-Affinity Chromatography (IMAC). It is commonly used method for high throughput purification of produced Ab via binding of 6X-His tag residues available on the N terminus or C terminus of protein to Nickel. Nitroloacetic acid (NTA) beads are used to bind the Nickel to agarose beads. Simply Ni-NTA column chromatography is based on the absorbing of protein of interest onto the column by mixing the sample with the beads. In order to remove low affinity bound or unbound proteins, Imidazole and phosphate are added in a low concentration and the elution of protein of interest from the NTA beads is achieved by addition of Imidazole in a higher concentrations

1.5. Gold nanoparticles

Gold colloids have been used to stain glass since ancient times and it was investigated in detail by Michael Faraday in 1857 (Thompson, 2007). Currently the nanoscale properties of gold nanoparticles (GNPs) have attracted more attention and they are used in different fields like electronics (Rothenberg et al., 2005), optics (Yao et al., 2000) and biosensors (Aslan et al., 2004, Stuart et al., 2004). GNPs under the influence of an interacting incident light, the surface phenomenon i.e., SPR has been related to their unique optical properties (Liz-Marzan, 2006). The nanoparticle surface related properties are easily affected by size, shape and their surrounding dielectric environment (Link and El-Sayed, 1999). In addition to their optical property, GNPs are resistant to oxide formation under ambient conditions. The nature of their surface chemistry promotes easy and controlled attachment of other molecules especially those with thiol functionalities. Following their exciting properties and the controllable morphology, GNPs based bioconjugates are found to be good candidates for biomedical applications. Their biocompatibility, high stability, ease of characterization (Nie and Emory, 1997, Rosi et al., 2006, Sperling et al., 2008, Grzelczak et al., 2008) and non toxicity are some of their special properties that promote their diverse application. A number of diagnostic methods including selective cell and nuclear targeting, site-specific diagnosis and treatment of ailments involve the use of GNPs as labels and vectors.

1.5.1. Gold Nanoparticles for biosensing

Enzyme labels have been used extensively in immunoassays such as microtiter ELISA, chemiluminescent assays, and flow-through or vertical flow membrane immunoassays. A primary advantage of using enzyme labels is that the potential amplification can be achieved. However, they are less popular in LFIA, especially self-contained assays. Colloidal gold based tests can be completed in one step as compared to ELISAs (Nara et al., 2010). Gold probes do not require incubations, washing steps and enzymatic reactions to generate a signal. It was shown that signalling efficiency becomes higher by increased sized GNPs (Paek et al., 2000). These characteristics significantly shorten the analysis time and make it ideally suited for on-site testing by untrained personnel. Since Abs are labeled with colloidal gold particles instead of enzyme, the substrate is no longer needed in the reacting system for colloidal gold immunochromatography. This is the reason why colloidal gold particles have been gradually applied in immunoanalysis and biosensor developments (Deng et al., 2000, Sithigorngul et al., 2007). Biosensors using GNPs have been designed previously (West and Halas, 2003, Pavlov et al., 2004). The principle governing such methods is the interactions of biomolecule functionalized GNPs with their targets resulting in aggregation. This has been used as means of identifying analytes in solutions. Aggregation causes the changes in the optical absorption behavior of the particles. These changes could easily be monitored by naked eye. GNPs or GNPs-aggregation based immunoassays have been performed based on antigen-Ab interactions for protein detection (Dykman et al., 2005).

LFIAs based on GNPs/Ab conjugates have become useful innovation in nanotechnology. An important advancement in the past was the replacement of the common organic dyes used in LFIA fabrication with GNPs. Colloidal gold is the most widely used label today in commercial LFIA for many reasons (Chandler et al., 2000). It is fairly easy and inexpensive to prepare in the laboratory. The color is intense, and no development process is needed for visualization. Unfortunately, there are some basic limitations of the present generation of LFIA kits. For instance, assays have varying sensitivity levels with respect to their target agents (Peruski and Peruski, 2003). Storage time of test results is limited due to poor stability of GNPs

conjugates used for the fabrication. In summary, the detection limit of analytes from μM to zeptomolar is achieved by GNPs based sensors (Saha et al., 2012) and it can be enhanced for the lower detection limit of different agents with nanoscale sensors.

1.5.2. Synthesis of metallic nanoparticles

A common synthetic route of GNPs is the aqueous reduction of $HAuCl_4$ by sodium citrate at boiling point (Narayanan and El-Sayed, 2005, Frens, 1973). Other reducing agents like borohydrides and amines have been used (Wilson et al., 2002, Mayer and Mark, 1998). Particles synthesized by citrate reduction are nearly monodisperse spheres whose sizes could be controlled by the initial reagent concentrations (Hostetler et al., 1998, Pillai and Kamat, 2004). These particles' surfaces are covered with negatively charged citrate layers characterized by Zeta potentials. Figure 7 depicts the citrate molecules on spherical GNPs (Wulandari et al., 2008). They have optical properties characterized by their plasmon absorbance band in the visible region of the electromagnetic wave, i.e., 16 nm particles have absorption peaks at about 520 nm. Interactions between particles in a suspension greatly affect their stability. Such interactions could either be stabilizing or destabilizing in nature. For example, Van der Waals forces draw colloidal particles together while electrostatic forces cause repulsion of particles from one another. If the attractive forces between particles in suspension become greater than the repulsive forces which means that if the distance between particles is smaller than the sum of their electric double layer distances, the particles will flocculate and precipitate.

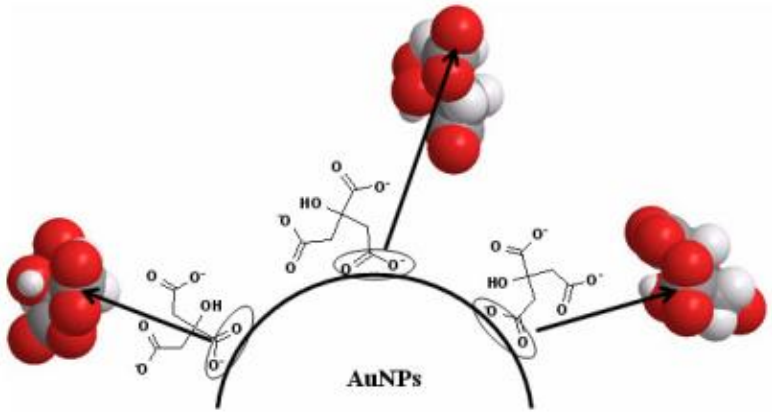


Figure 7. Anchoring of citrate molecules on GNPs. (Taken from Wulandari et al., 2008)

1.5.3. Bioconjugation of Gold Nanoparticles

Metal colloidal particles are stable with their conjugated parts compared to the unbound forms. If the sodium chloride as an electrolyte presents in the solution, repulsive and attractive forces between the particles are imbalanced due to the masking of negative charge of colloidal solution (Derjaguin and Landau, 1941, Verwey and Overbeek, 1948). This is resulted with collapsing of gold particles after adsorbing of one particle onto another and visualization of this phenomenon is seen as color change of colloids. As an example, the red color of GNPs with different sizes is changed to blue or grey when agglomerated. However, in the presence of proteins, they adsorb onto gold particles and help preventing their aggregation by inhibiting the binding of other gold particles. The complex of colloidal gold-protein was studied by Johne et al., (1995) firstly, in the BIAcore biosensor. Adsorbing of the other components of the solution is the major disadvantage of the affinity of colloidal particles for proteins resulting with undesired labelling. Therefore, inert macromolecules, e.g. polyethylene glycol, gelatin or BSA must be used for stabilization of metal conjugates in a colloidal form.

Several parameters must also be considered for preparing the stable GNPs-protein complex in a good quality. First, pI (the isoelectric point) of the protein, second, pH value of the reaction, third, the amount of the protein loaded onto colloidal particles.

To achieve the protein adsorption on metal surfaces optimally, pH value of the buffer systems should be close to the pI of the proteins as the adsorption is decreased if pH is away from pI (Thobhani et al., 2010).

The coupling and functionalization of nanoparticles with proteins is done by a large body of protocols exists in the literature. These include electrostatic interaction, ligand recognition, metal-mediated complexation, chemisorption, and covalent binding through bifunctional linkers (Collings and Caruso, 1997, Rao, et al., 1998, Scouten et al., 1995). Abs and other proteins contain amino acid polymers with a number of reactive side chains which could be utilized for attachment. Alternative to these intrinsic reactive groups, specific reactive moieties could be generated or introduced onto the molecule by chemical modification. These introduced groups are consequently used as "handles" for attaching other molecules or particles.

1.5.3.1. Physical interaction

This is a simple method to form GNPs-Ab conjugates through electrostatic interactions of Abs with charged particles' surfaces. It was suggested that electrostatic interaction between proteins and GNPs depends on three separate but dependent phenomena, (i) ionic attraction between the negatively charged gold and the positively charged Ab. (ii) hydrophobic attraction between the Ab and the gold surface. (iii) dative binding between the gold conducting electrons and amino acid sulfur atoms of the Ab (Hayat, 1989).

1.5.3.2. Chemical interaction

The problems facing electrostatic coupling method like conjugate instability and IgG inactivation could be solved by using covalent conjugation methods (Caruso, 2001). This is achieved in a number of ways like chemisorption via thiol derivatives and through the use of bifunctional linkers. The chemisorption of proteins onto a metal particle surface can be proceeded by through cysteine residues present in the protein and chemical way in the presence of 2-iminothiolane (Droz et al., 1996, Ghosh et al., 1990).

1.6. Lateral Flow Immunochromatography (LFI)

LFIA also known as immunochromatographic strip tests were described in 1960s (Kohn, 1968). They have been a popular platform for rapid immunoassays since their introduction in the mid-1980s and they are developed by Ab, aptamer and nucleic acids (Laderman et al., 2008, He et al., 2011, Ang et al., 2012). They are simple diagnostic test kits commonly based on a nitrocellulose membrane (NC) matrix. They are used to detect the presence or absence of a target analyte in a sample. LFIAs are expressed in different forms, ranging from Immunochromatography Assay (IA), Lateral Flow Assay (LFA), Lateral Flow Device (LFD), Dipstick-Assay, Point of Care (POC) to Bed Side Test, depending on their formats. A common feature is that they are all based on accumulation concepts (Ijeh, 2011). The lateral flow (LF) format is so versatile that manufacturers of rapid immunoassay tests have developed LF assays for almost any situation where a rapid test is required. Today this technique has found growing applications in proteomics, therapeutic monitoring, infectious and chronic diseases, non-human applications such as animal health or veterinary, agriculture, biowarfare, environmental health, and safety and food industry **(**O'Farrell, 2009). In these circumstances, rapid tests are used to screen for pathogens and toxins in raw materials, the manufacturing environment and the finished product. Besides, water utilities test drinking water for chemical toxins, metals, and pathogens. Rapid test systems are also used to assess progress in environmental remediation of soil and ground water pollutants. For medical diagnostics they are often used for tests either at homes or in the laboratories to detect the analytes including pathogens and toxins (Ayong et al., 2005, Delmulle, 2005, Mettler et al., 2005), and body fluids.

Compared with other analysis methods, the immunochromatography strip test has many advantages. The reasons for the continued popularity of LF tests are established mature technology, relative ease of manufacture, processes already developed, easily scalable to high volume production, stable shelf-lives of 12–24 months often without refrigeration, minimal operator dependent steps and interpretation, handling small volumes of multiple sample types, integration with onboard electronics, reader systems, and information systems, having high sensitivity, specificity, good stability, relatively low cost and short timeline for development and approval, market presence,

and acceptance minimal education required for users and regulators (O'Farrell, 2009). However, traditionally designed LFIAs have some drawbacks such as unclear patent situation, miniaturization of sample volume requirements below microliter level, integration with onboard electronics, sensitivity issues in some systems, and test to test reproducibility. Besides, the imprecision that is due in part to the subjective visual interpretation of test results in quality and performance of materials is another disadvantage. Despite issues of perceived or real performance limitations, LFIAs have achieved broad penetration in a variety of markets. Figure 8 lists the market segments in which LFIAs are already in production or known to be in development.

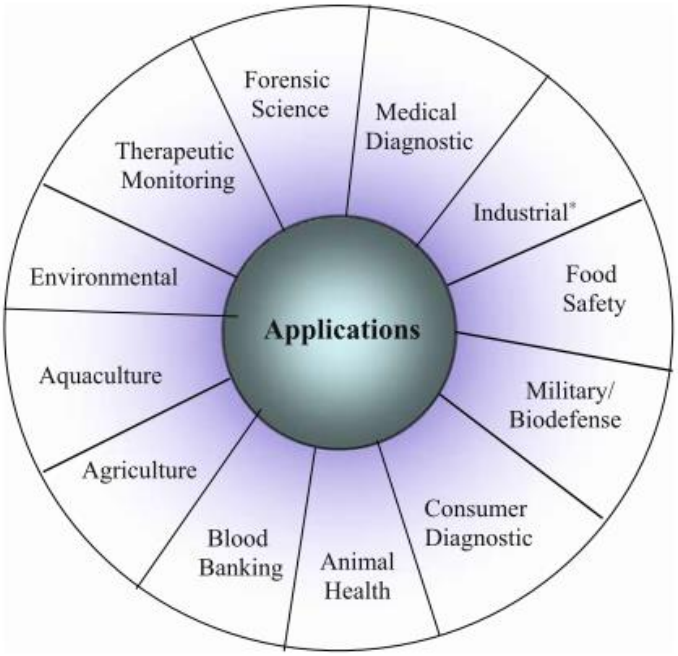


Figure 8. Market segments for LFIA and other field use technologies. (Taken from O'Farrell, (2009) *includes applications such as QC, product identification, environmental monitoring, safety

Some of the more common immunochromatographic LF strip tests currently available can be listed as for pregnancy and infections including Streptococcus, Chlamydia, human immunodeficiency virus (HIV) and Hepatitis C virus (HCV) (Zhang et al., 2009). The newest LF test on the market is Inverness/Matritech's BladderChek Test POC test for bladder cancer.

1.6.1. Manufacturing of Lateral Flow Test Strips

The general manufacturing process for production of typical test strips includes the preparation of colloidal gold conjugates, application of reagents onto the membrane and pads, lamination of the strip membranes onto a support backing, cutting the prepared master cards into strips of defined length and width, and strip packaging (Figure 9).

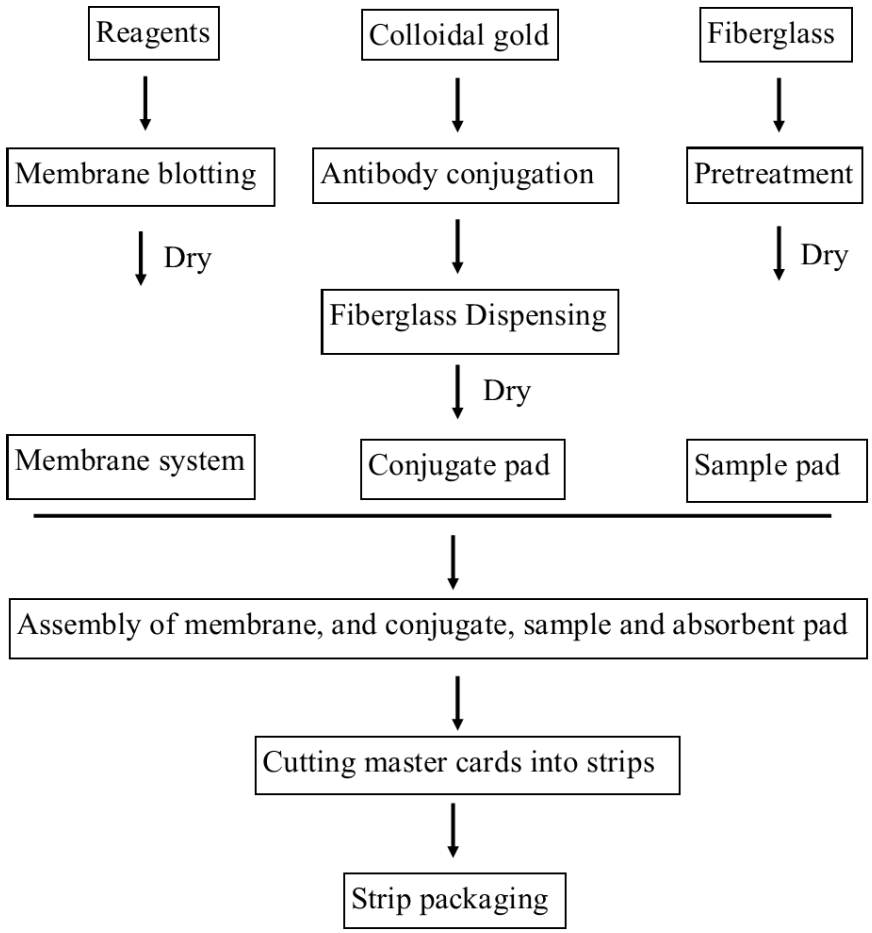


Figure 9. Represantation of a LF strip manufacturing process. (Taken from Zhang et al., 2009)

The most common plastics used for LFIA tests are polystyrene and polyester. The test sample is applied onto the sample pad. Conjugate pad contains Abs, which are specific

to the target analyte and usually conjugated to coloured particles such as GNPs and latex beads. Anti target Abs are immobilised in a line across the membrane which is a hydrophobic nitrocellulose or cellulose acetate as a test line. It has an additional control line containing Abs specific for the conjugate Abs. The components of the strip are usually fixed to an inert backing material and may be presented in a simple dipstick format or within a plastic casing with a sample port and reaction window showing the capture and control zones (Peruski and Peruski, 2003). After the test sample is applied, it flows along the nitrocellulose membrane via capillary action, on which it encounters a colored reagent, e.g. GNPs/Ab conjugates applied and continues through the zones containing immobilized Abs. Depending upon the analytes present in the sample the colored reagent can become bound at the test line. Providing that the test strip and the test procedure are correct, the control line is always visible. If no colored capture line or only a red color at the test line appears, the strip is invalid, and the test should be repeated using a new strip (Song et al., 2011, Tripathi et al., 2012). Besides, the test result is positive when both the test and control lines are red color (Figure 10).

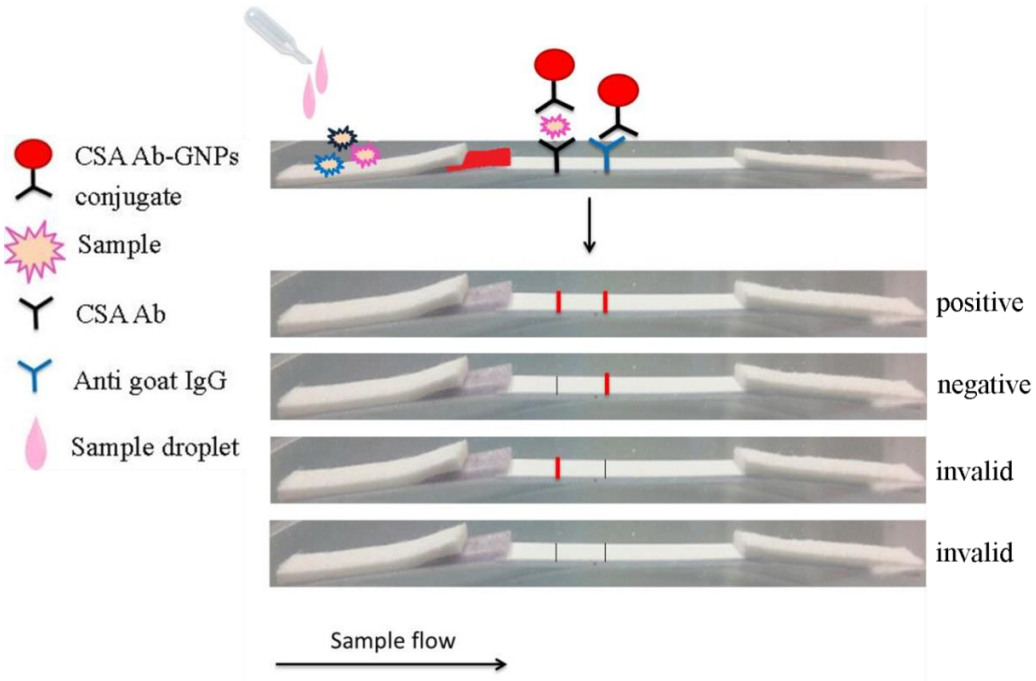


Figure 10. Schematic diagram of the immunochromatographic test strip principle for the dipstick assay. CSA Ab: Common structural antigenic antibody, GNPs: Gold nanoparticles

For the smaller analytes the competitive format of LF assay can be used (Zhang et al., 2006b, Molinelli et al., 2009). Test procedure and principle of competitive format is that suitable volume of sample extract is added onto the sample pad, and the solution migrates toward the absorbent pad. When sample analyte is absent from the sample, all of the detection reagent would be trapped by capture reagent to form an easily visible test line. When sample analyte is present in the sample, it competes with the immobilized capture reagent for the limited amount of competitive detection reagent. Thus, it means that the more sample analyte in the sample, the weaker the test line color.

1.6.2. Market Size and Trend of Lateral Flow Test Strips

Immunoassays have been used in hospitals, laboratory medicine, and research since the mid-1960s. The first commercial application of immunochromatographic strip tests is a pregnancy test introduced in 1988 (Posthuma-Trumpie et al., 2009). If the target analyte and necessary Abs are available, assay development costs can be as low as $30,000 or not higher than $100,000. Further, prototype test kits can be available for clinical trials in as little as 4 months. Once developed and tested, test kits can be manufactured for $0.10–$3.00 per test.

The world market for LF based tests (Kalorama Information, 2006) was estimated at $2,270 million in 2005 with a compounded annual growth rate (CAGR) of 10%, and it was reached $3,652 million in 2012. This estimate included LF based tests used in human and veterinary medicine, food and beverage manufacturing, pharmaceutical, medical biologics and personal care product production, environmental remediation, and water utilities. Biowarfare agents and pathogens such as anthrax, smallpox, avian influenza, and other potential biological weapons are not included in this table since they are no use in routine and developmental process of them is still proceeding. The US market accounts for $1,005 million (50%) and the European market for approximately $804 million (40%) of the worldwide market for LF clinical tests. Japan and Asia represents 5% ($100 million) and the ROW accounts for the remaining 5% of the market (Rosen, 2009). Table 4 demonstrates the revenue of LF test sales.

Table 4. World Lateral Flow Test Sales Revenue (US$ Million). (Taken from O'Farrell, (2013)

	2010	% Market	2015	% Market	% CAGR	No. of Companies
Clinical	2990	89	4100	89	7	At least 100
Veterinary	280	8	420	9	8	At least 50
Food and beverage	50	1	95	1	14	Approx. 20
Pharma/biologics	20	0.6	35	1	12	Approx. 20
Environment	10	0.2	12	0.2	4	At least 50
Water utilities	10	0.2	13	0.2	5	Approx. 20
	3360	100	4675	100	7	

1.6.3. Antibodies for Lateral Flow Strips

Abs suitable for LFIA are available from a number of commercial sources (Linscott, 2008). Various kinds of Abs generetad by different ways such as hybridoma or recombinant protein technology can also be adaptable to the LF assay. Although monoclonal or polyclonal Ab based immunochromatographic test strips are commonly in use, development of scFv based tests (Wemmer et al., 2010) is highly limited. Frequently, commercial Abs can be obtained prequalified by the vendor in pairs suitable for sandwich assays. These pairs are most readily available for relatively common and high volume assays, such as tests for pregnancy, infectious disease, cardiac markers and malignancies. Similarly, Abs suitable for use in competitive assays, such as hormones, therapeutic drugs, and drugs of abuse are available. Abs can be directly immobilized onto GNPs (Shen et al., 2007) and then they are applied to the developed strip assays. The first use of an Ab with colloidal gold reagent for a diagnostic immunoassay was reported in 1981 (Leuvering et al., 1981). It is also possible to obtain some of those Abs which are already conjugated to colloidal gold or latex particles.

1.7. Aim of the Study

Fast and sensitive detection of food pathogens by using commercial and engineered Abs has been proposed. One of the main objectives of this research was to develop the LF test strips based on GNPs in order to detect Salmonella and *E.coli* O157:H7 species. Second purpose of this study was to generate the scFv of Salmonella specific se155-4 Ab and to test its binding affinity and specifity to target antigens by ELISA, and finally

to adopt the scFv to the LF strip technology for the purpose of making self produced Ab based detection platform for Salmonella. The last goal of this research was to detect Salmonella by engineeringly produced humanized full length IgG, fusion Ab, form of se155-4 Ab by common and magnetic based ELISA in a very low cell number as a detection limit.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

2.1.1. Chemicals, Kits and reagents

Isopropyl β-D-1-thiogalactopyranoside (IPTG) (Life Technologies); superior broth media (AthenaES); SeaKam GTG Agarose (Lonza); EDTA (Duchefa Biochemie); dialysis tube (Sigma D-30); formaldehyde (37%); SOC outgrowth medium and 2XYT medium, LPS from *Salmonella enterica serotype typhimurium* and Anti-polyHistidine−Peroxidase antibody (Sigma); 1kb DNA ladder; affinity purified antibody to Salmonella common structural antigen (CSA) (01-91-99), *S. typhimurium* positive control cells (50-74-01), affinity purified antibody to goat IgG (01-13-06), affinity purified antibody to *E.coli* O157:H7 (01-95-90-MG) and *E.coli* O157:H7 positive control cells (50-95-90) (KPL); His pur^{TM} Ni-NTA Resin, MES SDS Running buffer, Gelcode Blue Stain reagent, Costar 3590 ELISA microplate, Spectra BR^{TM} multicolor broad range protein ladder, Ultra TMB_ELISA substrate solution, Slide-A-Lyzer G2 Dialysis Cassettes and 10K MWCO (Thermo Fisher scientific); LB medium; Kanamycin (50µg/ml final); Ampicillin (0.1mg/ml final); pET28(+a) (Novagen); Salmonella ELISA Kit (Abnova); QIAprep Spin Miniprep Kit (Qiagen); Bis-Tris Gel; Accuprime Pfx DNA polymerase and Pfx Accuprime rxn mix (Invitrogen); Filters cups (Thermo scientific, Nalgene); sterile syringe filter (VWR); Cellulose sample&absorbent pad, roll (C083), Glass fiber conjugate pad (G041) and NC membranes (HF240MC100, HF180MC100, HF120MC100, HF075MC100) (Millipore); skim milk powder (OXOID); Laemmli sample buffer (Biorad) and of 2-Mercaptoethanol (MP Biomedicals) were used and all the gene pieces including primers and geneblocks were purchased from Integrated DNA Technologies (IDT).

ExpiFectamine™ 293 Transfection Kits, Opti-MEM® I Reduced Serum Medium (31985-062), Expi293 Expression medium A14351-01, 1000ml (Invitrogen-Gibco by Life Tech.); ProMag™ 1 Series • COOH (Bang laboratories, Inc- PMC1N); SNAP capture magnetic beads (NEB-S9145S) and BG-GLA-NHS substrate (NEB S9151S); and S. *typhimurium* LPS factor 4 Ab (MBS219614, Mybiosource) were used.

2.1.2. Bacterial strains

SHuffle® T7 Express Competent *E. coli* cells, DH10B electrocompetent cells and C3029 cells were purchased from NEB. *Salmonella enterica subspecies, serovar typhimurium* 14028s & LT2 were a gift from Dr. Stephen Trent (Molecular Genetics and Microbiology, The University of Texas at Austin), *S. bongori, S. ohio, S. newport* were kindly provided by Dr. Shelley Payne's lab (Molecular Genetics and Microbiology, The University of Texas at Austin). Dry soil bacteria sample was prepared from the common campus area of Middle East Technical University by collecting and culturing soil. Other used bacteria were given at Table 5.

Table 5. Bacterial strains used for testing developed LF strips

Strains	issue	Source
S. typhimurium	-	NanoBiz R&D lab
S. enteritidis	-	NanoBiz R&D lab
S. infantis	-	NanoBiz R&D lab
B. cereus	-	NanoBiz R&D lab
dry soil bacterial culture	-	METU campus, dry soil
S. enteritidis (1,2,3)	isolate	Ankara University Faculty
S. infantis (1,2,3,4,5,6,7)	isolate	Of Veterinary Medicine Ankara University Faculty of Veterinary Medicine
E.coli	-	NEB
***E. coli* O157:H7**	heat treated positive control cells	KPL 50-95-90
V. chloreae	heat treated positive control cells	KPL 50-90-02
S. typhimurium	heat treated positive control cells	KPL-50-74-01

2.1.3. Instruments

NanoDrop 2000 UV-Vis Spectrophotometer (Thermoscientific) was used for quantifying of either DNA or protein samples. UV-Vis Spectrophotometer

(Thermoscientific) was used to get spectra from GNPs and conjugates. Cell density meter (Biochrom WPA) was used for measuring the OD_{600} value of bacteria. Malvern Zeta Sizer was used for measuring both the size and Zeta potentials of GNPs and conjugates. Transmission Electron Microscopy (TEM) was used to analyze the size and shape of synthesized GNPs. Branson Sonifier 450 homogenizer was for the sonication of bacterial cells and Beckman coulter Avanti J26 XPI was for the spinning of cells. Fast protein liquid chromatography (FPLC) (Amersham, Frac 920) was for further purification of generated scFv and humanized fusion Ab. Microtiter plate reader (Tecan Infinite M200 PRO) was used for ELISA, Multiscan plate reader was used to get spectra from gold conjugate, Incubator-Shakers were also for the bacterial growth.

2.2. Experimental strategy

This study comprises two main parts, developing immunochromatographic test strips based on full length and scFv Ab using GNPs for food pathogens detection and recognition of Salmonella with engineered scFv and fusion Ab by common and immunomagnetic ELISA (Figure 11).

Developing the rapid detection tools for food pathogens is crucial to ensure the health of the organisms. LFIAs provide to users simplicity and point of care facility in a very short time without needed trained personel, and they are also popular in market trend. Since this technology have potential usage in almost every industries including medicine, veterinary, agriculture, etc. Ab based LFIA platform was developed in order to recognize the Salmonella and *E.coli* O157:H7 in the first part of the study. After synthesizing the GNPs with desired sizes, they were coupled with either commercial Abs against to Salmonella and *E.coli* O157:H7 or generated scFv specific to Salmonella.

scFvs play a significant role in Ab based detection technologies. Because their production is possible with non-mammalian cells by time saving process and they retain antigen binding capacity in a recombinant form. Fusion Abs are also popular in the labelling process for imaging and detection technologies. Therefore, in the second

part of the study, common ELISA were performed in order to determine the binding efficiency of both engineered scFv and fusion Ab to Salmonella serogroup B&D, and immunomagnetic ELISA was also applied to make a very sensitive detection of *S. typhimurium* by produced fusion Ab.

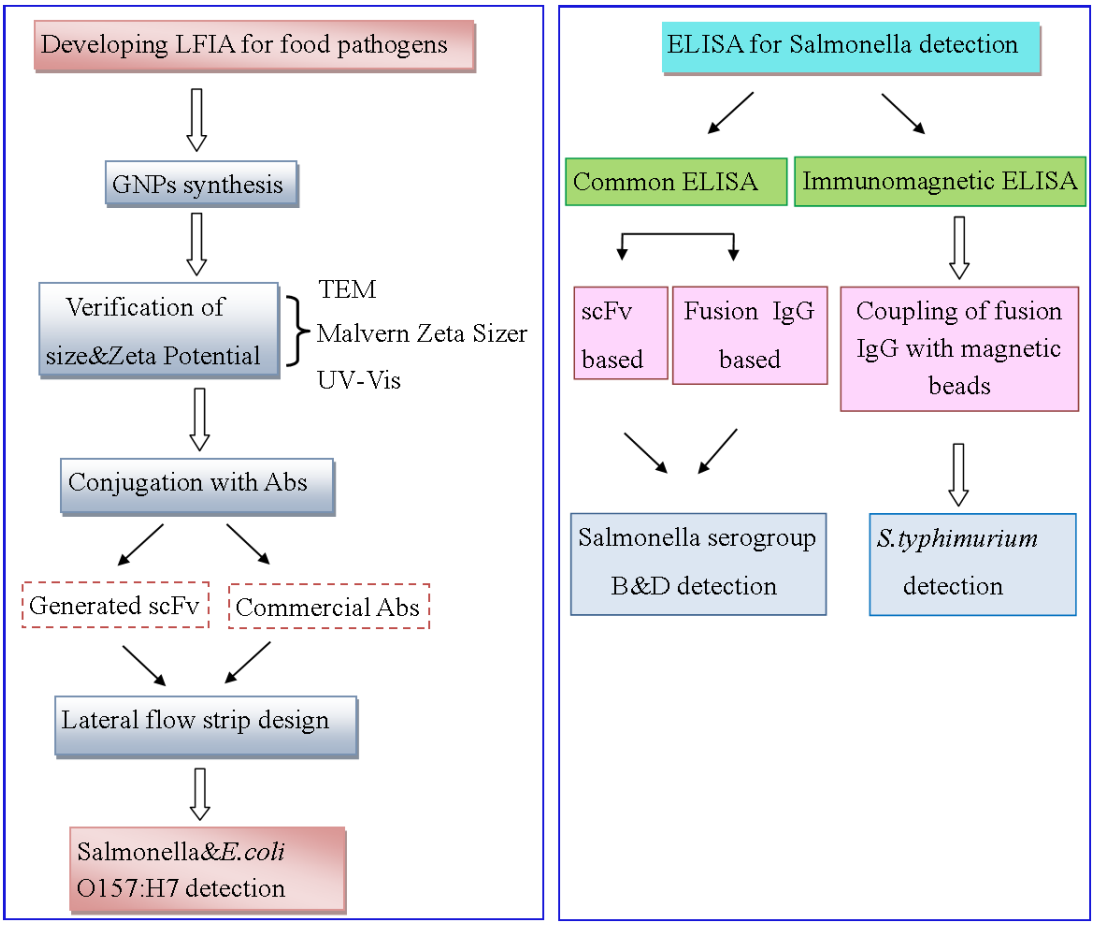


Figure 11. Overview of experimental strategies and methods for recognizing of food pathogens, Salmonella and *E.coli* O157:H7, in this study. LFIA: Lateral Flow Immunochromatographic Assay, GNPs: Gold nanoparticles, TEM: Transmission Electron Microscopy, UV-Vis: UV-Visible Spectroscopy, Abs: Antibodies, scFv: Single chain variable fragment

2.3. Methods

2.3.1 Bacterial growth

S. infantis (FE), S. paratyphi B, S.montevideo, S. telaviv, S. kentucky were cultured in BHI medium. *S. typhimurium, S. enteritidis, S. infantis* and dry soil bacteria were cultured in TSB medium and other remained bacteria were in LB medium. Their OD_{600} value was measured with spectrophotometer.

2.3.2. Generation of recombinant antibodies

2.3.2.1. Production of Single Chain Variable Fragments

The amino acid sequences of variable fragments of se155-4 Ab were obtained from the Genbank. Accession numbers are AAB30721.1 and AAB30722.1 (Bundle et al., 1994b) for heavy and light chain variable regions, respectively. Aminoacid and nucleotide sequences of variable fragments were given at Table 6. The gene block of scFv with the linker sequences was also shown in Table 7.

Table 6. Aminoacid and nucleotide sequences of variable fragments of se155-4 Ab

Heavy chain variable region of Se155-4 Ab
EVQVQQSGTVLARPGASVKMSCKASGYTFTNYWMHWIKQRPGQGLEWIGAIYPGNSATF YNHKFRAKTKLTAVTSTITAYMELSSLTNEDSAVYYCTRGGHGYYGDYWGQGASLTVSS AK GAAGTTCAAGTGCAGCAGTCTGGAACCGTGCTTGCGAGACCAGGCGCAAGTGTTAAGA TGTCCTGTAAAGCTTCCGGGTATACTTTTACGAACTATTGGATGCATTGGATAAAACAA CGCCCTGGGCAGGGGCTCGAATGGATTGGCGCTATATACCCAGGGAACAGCGCGACTT TTTATAATCACAAGTTTCGGGCGAAAACCAAACTCACAGCTGTCACAAGCACGATAAC CGCTTATATGGAGTTGTCTAGCTTAACGAACGAAGACTCTGCCGTATACTATTGTACAC GGGGAGGGCATGGTTATTATGGGGACTATTGGGGCCAGGGTGCGTCACTTACGGTCTC GAGTGCCAAG
Light chain variable region of Se155-4 Ab
QAVVTQESALTTSPGETVTLTCRSSTGTVTSGNHANWVQEKPDHLFTGLIGDTNNRAPGVP ARFSGSLIGDKAALTITGAQPEDEAIYFCALWCNNHWIFGGGTKLTVLGQPK CAAGCGGTGGTCACTCAAGAGTCCGCCTTGACCACCTCACCTGGCGAAACAGTCACTC TGACATGTCGGAGCAGCACAGGGACGGTGACCTCAGGCAATCATGCAAATTGGGTCCA GGAGAAGCCTGATCACTTATTCACCGGGTTGATCGGAGATACGAATAACAGGGCTCCA GGAGTGCCTGCTCGCTTTTCGGGGAGTTTAATTGGAGACAAGGCAGCCCTGACAATTA CCGGCGCACAGCCTGAGGACGAAGCCATATACTTTTGCGCCTTATGGTGCAATAATCA CTGGATTTTCGGGGGCGGAACCAAGCTCACTGTGCTGGGCCAACCAAAG

Table 7. Gene block of scFv with linker sequences

CTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATCATATGAAGAAAACGGCCATC
GCTATTGCCGTGGCGCTGGCTGGATTTGCAACAGTGGCACAGGCGGAGGTACAGGTCC
AGCAGTCCGGCACCGTCCTTGCGCGCCCGGGCGCTTCCGTGAAAATGAGCTGTAAAGC
GAGTGGGTACACATTTACTAATTACTGGATGCACTGGATTAAACAGCGTCCTGGCCAA
GGTTTGGAATGGATCGGAGCGATTTATCCTGGCAATAGCGCTACCTTTTACAATCATAA
GTTTCGCGCTAAAACCAAACTTACCGCCGTTACCAGCACTATCACAGCGTATATGGAA
CTGTCTTCGCTGACAAACGAAGACAGCGCTGTTTACTATTGCACCCGCGGCGGACACG
GCTATTATGGTGATTACTGGGGCCAGGGGGCCTCGTTGACGGTATCGTCCGGCGGGGG
AGGCAGTGGGGGCGGCGGTTCTGGTGGTGGTGGTTCCCAGGCAGTAGTGACACAGGA
GAGTGCCCTGACCACCAGCCCGGGTGAAACCGTAACACTCACTTGTCGCAGCAGCACA
GGTACTGTGACATCCGGTAACCATGCGAATTGGGTTCAGGAAAAACCGGATCACCTGT
TTACTGGCCTGATTGGAGATACGAACAATCGCGCGCCTGGCGTGCCAGCCCGCTTTAG
CGGCAGCCTGATCGGGGATAAAGCAGCTCTGACCATCACAGGCGCACAACCAGAAGA
TGAAGCAATCTATTTTTGCGCGCTGTGGTGCAACAATCATTGGATCTTTGGTGGTGGAA
CAAAACTGACCGTTCTGGGACAGCCGAAAGGATCCCACCACCACCACCACCACTGATG
A

The construction of pET-28a (+) vector (Figure 12) for inserting the scFv gene block was carried out in the Ellington Lab, The University of Texas at Austin.

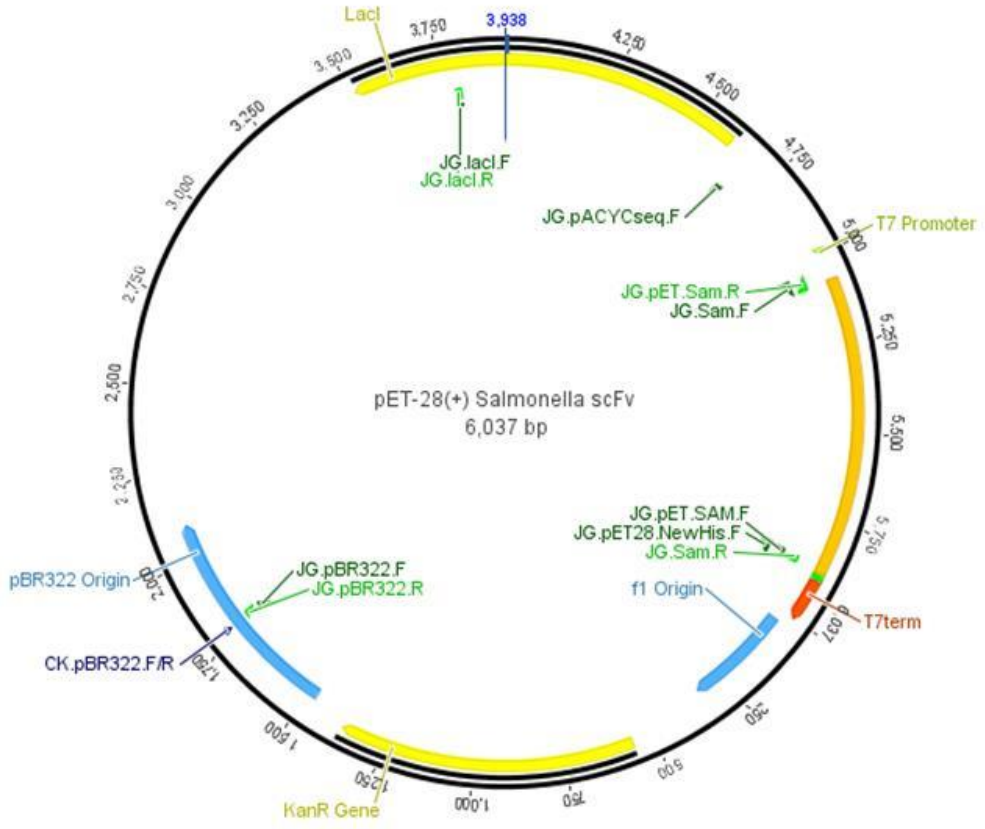


Figure 12. scFv construction of se155-4 Ab using pET28a (+) vector

In order to build the Salmonella scFv, vector and geneblock PCR were done, initially. The amplified regions of both vector and gene block were carried out by designed forward&reverse primers (Table 8). Primer sets, reagents used for PCR and PCR cycling conditions were given at Table 9, Table 10, and Table 11 respectively.

Table 8. Primers used for scFv building

JG.Sam.F	CTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATCATATG
JG.Sam.R	TCATCAGTGGTGGTGGTGGTGG
JG.pET28.NewHis.F	GGATCCCATCACCACCATCATCACTGATGAGATCCGGCTGCTA ACAAAGC
JG.pBR322.R	CGCCTTATCCGGTAACTATCGTCTTG
JG.pBR322.F	CAAGACGATAGTTACCGGATAAGGCG
JG.lacI.R	GCGTTGGTGCGGATATCTCGGTAG
JG.lacI.F	CTACCGAGATATCCGCACCAACGC
JG.pET.Sam.R	CATATGATATCTCCTTCTTAAAGTTAAACAAAATTATTTCTAG

Table 9. Primer sets used for PCR rxn

Vector PCR	Forward	Reverse	bp
1	JG.pET28.NewHis.F	JG.pBR322.R	1966
2	JG.pBR322.F	JG.lacI.R	2036
3	JG.lacI.F	JG.pET.Sam.R	1287
Gene PCR	Forward	Reverse	bp
G1	JG.Sam.F	JG.Sam.R	871

Table 10. Reagents used in PCR rxn

Reagent	**Stock concentration**	**Final concentration**	**Volume (µl)**
Buffer*	10 X	1 X	5
Template**	1 µg/µl	10 ng	0.5
JG.Sam.F primer	20 µM	0.4 µM	1
JG.Sam.R primer	20 µM	0.4 µM	1
Polymerase***	5 U/µl	0.05 U/µl	0.5
dH_2O			42
Total			50

* Pfx Accuprime rxn mix

** pET28(+a) plasmid DNA

*** Accuprime Taq DNA Polymerase

Table 11. Thermal cycling conditions used in PCR

		Temperature (˚C)	Duration	Number of cycles
Initial denaturation		95	3:00	1
Amplification	Denaturation	95	0:30	20
	Annealing	60 (1)*, 65 (2)*, 58 (3)*, 62 (G1)*	0:30	
	Extension	68	2:00 (1,2)* 1:00 (3,G1)*	
Final extension		68	4:00	1

* Codes coming from Table 9

After PCR reaction, the resulted DNA fragments were obtained by gel extraction according to the Promega manual. The concentration of DNA pieces was quantified using NanoDrop 2000, and stored at -20˚C for further use.

2.3.2.1.1. Transformation to *E.coli* cells

Gibson assembly reaction (Gibson, 2011) was performed to overlap the DNA fragments obtained from gel extraction. Then transformation was done using NEB 10-beta competent *E.coli* according to the protocol manufacturer supplied. In summary, 5 µl of the Gibson rxn was added into competent *E.coli* in order to transform the ligated products. After incubation on ice for 30 min, heat shock procedure was applied and finally 100 µl of outgrowth culture was plated onto LB agar plate including antibiotics Kan and incubated at 37˚C for overnight. On the following day, a single colony of transformed *E.coli* cells were picked up and inoculated into 5 ml of LB media added with Kan and allowed to incubate at 37˚C for overnight. Plasmids were isolated from bacterial pellets using the QIAprep Spin Miniprep Kit. The verification of plasmid DNA sequences was done by both PCR and sequence analysis which was performed at The University of Texas at Austin, Department of Molecular Bioscience. Then plasmid DNA was stored at - 20˚C after quantifying with NanoDrop 2000 UV-Vis Spectrophotometer.

2.3.2.1.2. Isolated Plasmid PCR

In order to make sure that synthetic scFv gene block is inserted in the plasmids, they were isolated firstly. Inserted gene block was amplified by PCR and then PCR products were loaded on 1% SeaKam GTG agarose gel with 6x orange loading dye and run in 1X TAE about 40 min using 136 V power supply. 1kb DNA ladder was used for the correction of the band sizes. Reagents for PCR and PCR cycling conditions are shown in Table 12 and 13, respectively.

Table 12. Reagents used in PCR rxn

Reagent	**Stock concentration**	**Final concentration**	**Volume (µl)**
Buffer*	10 X	1 X	5
Template**	50.5 ng/µl	0.5 ng	0.5
JG.Sam.F primer	20 µM	0.4 µM	1
JG.Sam.R primer	20 µM	0.4 µM	1
Polymerase***	5 U/µl	0.05 U/µl	0.5
dH_2O	-	-	42
Total			50

* Pfx Accuprime rxn mix

** Plasmid DNA

*** Accuprime Pfx DNA polymerase

Table 13. Thermal cycling conditions used in PCR rxn

		Temperature(˚C)	**Duration**	**Number of cycles**
Initial denaturation		95	3:00	1
Amplification	Denaturation	95	0:30	20
	Annealing	62	0:30	
	Extension	68	1:00	
Final extension		68	4:00	1

2.3.2.1.3. Expression of Single Chain Variable Fragments

The expression plasmids were transfected into SHuffle® T7 Express Competent cells. In brief, 1 µl of 50.5 ng/µl plasmid DNA was added into ~50 µl C3029 strain without vortexing and incubated on ice for 30 min. After that mixture was allowed to incubate for heat shock in water bath at 42˚C for 30 seconds, and placed on ice immediately after for 5 min. Then 950 µl of SOC medium was added into cells and they were incubated and rotated for 1 hr at 30˚C at ~250 rpm using New Brunswick Incubator. 100 µl of transfected cells were plated to LB agar plate containing Kan and incubated for 16 hr at 30˚C. On the following day five colonies were picked from the plate and grown in 5 ml of 2XYT (Sambrook et al., 1989) added with Kan separately, and they were incubated at 30˚C for overnight in the shaker. 10 ml of the overnight cultures as total volume was inoculated into 2 L of separate superior media added with Kan and incubated at 30˚C in a rotary shaker at 225 rpm. After the bacterial OD_{600} value reaches to 1.5, the anti-Salmonella scFv expression was induced by adding of 1 mM IPTG as a final concentration. After 24 hr growing period of cultures at 16˚C, 225 rpm, cultures were transferred into the centrifuge jars and pelleted at 4000RCF at 4˚C for 15 min. After discarding the supernatant the cell pellet was resuspended in 25 ml of ice cold equilibrate bufffer (pH: 7.4, 50 mM Phosphate, 300 mM NaCl, 20 mM Imidazole) on ice. The resuspended cells were transferred to sterile glass beaker for sonication. They were disrupted by 50% amplitude for 2 min as two times by 5 min time interval and then centrifuged at 40000x g at 4˚C for 30 min using Beckman coulter Avanti J26 XPI. The supernatant that is soluble fraction was filtered by 0.2 µm cellulose acetate into clean falcon tubes and held on ice for column purification after named as flow through (FT).

2.3.2.1.4. Purification of Single Chain Variable Fragments

All purification steps were done at 4˚C and buffers (Table 14) used for purification were autaclaved or filter sterilized. Briefly, 30 ml polyprylene columns were prepared with 2 ml of Ni-NTA solution and then prewashed with ultrapure water for two times. Firstly, columns were equilibrated with equilibrate or wash 1 buffer (W1) and the FT containing scFv comes from sonicated and centrifuged supernatant solution were

applied into columns. They were washed with wash 2 (W2) buffer and finally protein of interest was eluted with 5 ml of elution buffer (E). As a result of this, 10 µl of column purified scFv was loaded onto 4-12% Bis-Tris SDS Gel. It was run in 1X MES running buffer about 1 h at 140 V after incubating the aliquots at 95˚C for 3 min with loading sample buffer. Gel was stained with Gelcode Blue staining reagent for overnight and destained with ultrapure water about 4-5 hours. Multicolor broad range protein ladder was used as marker for the gel image analyses. The further FPLC purified scFv Abs were concentrated using 3000 MWCO centrifugal filter tubes. The concentration of obtained scFv was quantified by using Nanodrop 2000, and then it was stored at -80˚C or -4˚C in PBS for long term and short term storage, respectively.

Table 14. Buffers used for Ni-NTA column purification

	Equilibrate-Wash1 pH 7.4	**Wash2** pH 7.4	**Elution** pH 7.4
Na_2HPO_4 (0.5M)	4.05 ml	4.05 mL	4.05 mL
NaH_2PO_4 (0.5M)	0,95 mL	0,95 mL	0,95 mL
NaCl (5M)	3 mL	5 mL	5 mL
Imidazole (1M)	1 mL	2.5 mL	25 mL
dH_2O	41 mL	37.5 mL	15 mL
Total volume	50 mL	50 mL	50 mL
Final concentration	50mM Phosphate 300mM NaCl 20mM Imidazole	50mM Phosphate 500mM NaCl 50mM Imidazole	50mM Phosphate 500mM NaCl 500mM Imidazole

2.3.2.2. Production of Fusion Antibody

Construction of pSNAPf vectors for the heavy and SNAP-Tagged light chain of se155-4 Ab, transformation of them into the *E.coli* cells and plasmid isolation were performed in the Ellington Lab, The University of Texas at Austin.

2.3.2.2.1. Transfection and expression of fusion antibody

ExpiFectamine™ 293 Transfection Kit was used for transfection of mammalian cells. For that purpose final transfection volume was adjusted to 30 ml and the number of cells to transfect was 7.5×10^7 cells. The amount of plasmid DNA was determined as 30 µg. The amount of ExpiFectamine™ 293 Reagent was used as 80 µl which means

the volume of 2.7 µl ExpiFectamine™ 293 Reagent was used for per 1 µg of plasmid DNA transfected. The ratio of Ab chains was adjusted to 3:1 for the SNAP-Tagged IgL and IgH, respectively. Initally, cultured cells were tripsinized and the total number of cells needed were counted either under the microscope using hemocytometer or cellometer on the day of transfection. The appropriate volume of cells was added to erlenmayer shaker flasks and volume was brought up to 25.5 ml by adding pre-warmed Expi293 ™ Expression Medium for each 30 ml volume of transfection and the cells were returned to the incubator. Lipid DNA complexes were prepared for each transfection as following that 30 µg of plasmid DNA was diluted in 1.5 ml total volume of Opti-MEM® I Reduced Serum Medium and mixed gently. 80 µl of ExpiFectamine ™ 293 Reagent was also diluted in Opti-MEM® I medium to a volume of 1,5 ml and incubated at RT for 5 min after gently mixing. Then, the diluted DNA was added to the diluted ExpiFectamine ™ 293 Reagent in order to obtain a 3 ml of total volume. The mixture was incubated at RT for 20-30 min after mixing gently to form the complex of the DNA-ExpiFectamine ™ 293 Reagent. Then, 3 ml of complex was added to the each flask prepared by previously. Flasks containing a total volume of 28,5 ml was allowed to incubate in a 37˚C incubator with a humidified atmosphere of 8% CO_2 in air as shaking at 125 rpm. After about 16-18 hr incubation time, 150 µl of ExpiFectamine ™ 293 Transfection Enhancer 1 and 1,5 ml of ExpiFectamine ™ 293 Transfection Enhancer 2 were added to each flask, then waited for six days to obtain expressed SNAP-Tagged fusion Ab.

2.3.2.2.2. Purification of fusion antibody

The produced fusion Ab was purified with column purification as described in section 2.3.2.1.4 with slight differences. Buffers used for purification were also given in Table 15. All purification steps were performed at 4˚C and samples collected from columns were saved at each step for analytical gel. Briefly, 400 µl of Ni-NTA slurry was applied to the 5 ml columns, then they were equilibrated with 10 ml of cell culture medium. On the following, transfected cells were centrifuged at 500 RCF for 10-20 min at 4˚C. The supernatant was transferred to the clean falcon tube and added with 10X buffer (final 1X), then flew over to equilibrated columns and named as FT. The FT was

reloaded into column at least 3 times in order to increase the binding of protein onto Ni-NTA and washed with wash buffer once before applying 2-3 ml of elution buffer. 20 µl of the eluted fusion Abs and collected samples from each steps were analyzed by 4-12% Bis-Tris SDS gel with using 1X MES Running buffer. The gel was stained with Gelcode Blue Stain reagent at overnight and destained with ultrapure water. After making sure that fusion Ab is obtained succesfully, dialysis of purified Ab was also performed against 900 ml of 1X PBS at 4˚C for overnight using 10K dialysis cassettes with two times buffer change. After this step, excess amount of Imidazole was removed until the concentration of it reaches to 1mM using 10000 MWCO spin column by centrifugation performed at 3000 xg for 20 min. Purified fusion Ab was stored at 4˚C for further use or immediately coupled with magnetic beads after quantifying the concentration by using Nanodrop 2000.

Table 15. Buffers used for column purification of produced fusion antibody

10X buffer pH 8.0	**Wash Buffer** pH 8.0	**Elution Buffer** pH 8.0
500mM NaH_2PO_4	50mM $NaH_2PO_4.H_2O$	50mM $NaH_2PO_4.H_2O$
1.5M NaCl	300mM NaCl	300mM NaCl
100mM Imidazole	20mM Imidazole 0.05% Tween 20	300mM Imidazole 0.05% Tween 20

2.3.3. Development of Lateral Flow Strip Assay

2.3.3.1. Synthesis of Gold Nanoparticles and conjugation with antibodies

GNPs with different sizes were synthesized according to the citrate reduction method by making slight changes (Afonso et al., 2013) and conjugated with Salmonella CSA Ab, purified scFv and affinity purified Ab to *E.coli* O157:H7 using two different methods. The pH value of colloidal gold suspension was adjusted with 0.2 M K_2CO_3 or 1 M HCl. The evaluation of the size measurement of synthesized GNPs was performed by TEM, Malvern Zeta Potential instrument and UV-Vis spectroscopy.

Besides, Zeta potentials of both naked and conjugated GNPs were analyzed by Malvern Zeta Potential.

2.3.3.1.1. Synthesis of 16 nm GNPs and conjugation with Single Chain Variable Fragments

100 ml of 1 mM Hydrogen tetrachloroaurate (III) hydrate (99.9% Au) was boiled and 10 ml of 38.8 mM sodium citrate was added into the solution, then it was continued to boil about 10 min. On the following, it was cooled with constant stirring before filter sterilization and dialysis against water. Then, UV-Vis spectra was measured for max absorption spectrum of surface plasmons (λ_{max}) of synthesized GNPs. The conjugation of GNPs with dialyzed scFv against to PBS was initially started with the pH adjustment to 7.5-8.0-8.5-9.0-9.5 of GNPs. 40 µg/ml of scFv as final concentration was added into the each well containing 50 µl GNPs and after 15 min incubation absorbance value was read at λ_{max}. The stability and polydispersity constant of Ab/gold complex was evaluated by getting the ratio of absorbance at λ_{max}:580 nm and 600 nm:λ_{max} (Englebienne, 2000), respectively after adding 100 µl of 10% NaCl into solution. The optimal pH value was determined by the graph obtained from both the ratio of λ_{max}/580 nm and 600/λ_{max} absorbance value and also by looking at the red color of mixture which seems to the same as originally sythesized GNPs's red color. The minimum protein concentration desired for coating GNPs was determined in 96 well plate by adding of the Ab as 5-10-15-20-25-30-35-40-45-50 µg/ml for final concentration into each well. The absorbance value at λ_{max} was read after 15 min incubation and 100 µl of 10% NaCl solution was added into the wells. On the following of incubation for 45 min, it was reread at λ_{max}. Final decision for the optimal Ab concentration was made according to the λ_{max} decrease and also by looking at the color of mixture which is seen as the same red color of originally synthesized GNPs.

Conjugation procedure of 16 nm GNPs with scFv was as follows and called as method 1. The pH value of colloidal gold suspension was adjusted to 9.0 with 10 N NaOH and 11.1 µl of scFv at the predetermined optimum concentration, 20 µg/ml, was added into 989 µl GNPs and incubated for 1 hr with gentle shaking at RT. 10% BSA in 20 mM sodium borate (pH: 9.27) was added rapidly as 1/10 volume of conjugates, i.e.,100 µl,

into the mixture and allowed to incubate for 15 min before UV-Vis measurement. Mixture was pelleted at 16000x g for 30 min at 4˚C. Supernatant was discarded and pellet was washed with 1 ml wash buffer (WB, 20 mM sodium borate containing 1% BSA, pH: 9.27) for two times. Finally, pellet was resuspended in 100 µl WB and UV-Vis measurement was performed by Nanodrop. For negative control complexes, 750 µl of GNP was mixed with 75 µl 10% BSA in 20 mM sodium borate (pH: 9.27) and 11.6 µl PBS instead of scFv, then all remained steps were applied as the same with above and complexes were stored at 4˚C. To make scFv/5X concentrated GNPs conjugate, 4 ml of synthesized nanoparticles were centrifuged at 4500 rpm for 15 min at 8˚C using 10K ultracentrifugal filter tubes. Further 4 ml of GNPs was added into tube after discarding remained colorless volume and allowed to centrifugation at the same conditions. Finally, 2 ml of GNPs was added and after centrifugation, remained 450 µl pellet was added with 550 µl sterile water and called as 10X in 1 ml total volume. In order to make a conjugation with scFv, 500 µl of 10X GNPs was diluted as ½ ratio and called as 5X GNPs, then the coating procedure was applied as the same mentioned above. Finally pellet was used after resuspending in 500 µl WB (pH: 9.27).

2.3.3.1.2. Synthesis of 36 nm GNPs and conjugation with Common Structural Antigenic Antibodies

500 ml of 0.01% $HAuCl_4.xH_2O$ was boiled and 5 ml of 1% sodium tricitrate was added (Huo et al., 2006) into the solution. After changing the color of solution from black to reddish in a 2 min, it was allowed to further boiling about 10 min to complete the reduction. On the following, it was cooled with constant stirring before filter sterilization. After cooling, 0.05% sodium azide was added and UV-Vis spectra was measured for determining the λ_{max} of synthesized GNPs. The conjugate of GNPs with Salmonella and *E.coli* O157:H7 Ab was prepared according to the method 1 and method 2, respectively after dialysis of Abs against to 20 mM sodium borate (pH: 9.3) for 24 hr. Briefly, 0,1% EDTA was added into 80 ml distilled water and it was boiled about 2 min after putting the dialysis tube. Then, 2 ml of either Salmonella Ab or *E.coli* Ab with desired concentration was allowed to dialyze against to 200 ml 20 mM sodium borate (pH: 9.3) at cold room. Determining of the optimal Ab concentration for the conjugates was performed by applying the same procedure mentioned before except

for the adjustment of pH values and experienced protein concentrations which are 5.6-6.0-6.5-7.0-7.5-8.0-8.5-9.0 of GNPs and 1-2-4-5-8-12-16-20-24-28-32-36-40 µg/ml as final, respectively for Salmonella Ab. They were 6.3-8-8.5-9 and 5-10-15-20-25-30-35-40 µg/ml Ab for determining the optimal pH value and Ab concentration, respectively for *E.coli* Ab conjugation.

Conjugation procedure of GNPs with Salmonella Ab was performed according to the method 1 with slight changes. Briefly, 2 ml of the Salmonella Ab at the predetermined optimum concentration, 20 µg/ml, was added into 10 ml GNPs (pH: 9.0) rapidly and incubated for 30 min with gentle shaking at RT. 10% BSA in 20 mM sodium borate (pH: 9.3) was added rapidly as 1/10 volume of conjugates into the mixture and allowed to incubate for 15 min before UV-Vis measurement. Mixture was pelleted at 25000 RCF for 30 min at 4˚C. The supernatant was discarded and pellet was washed with 8 ml wash buffer (WB, 20 mM sodium borate containing 1% BSA, pH: 9.3) for two times. Finally, pellet was resuspended in 1 ml WB and UV-Vis spectrum was recorded. For negative controls, the same amount of GNPs was used for making different complexes (Table 16) without adding any Ab in order to make sure that there will not be non-spesific capturing on the strip assay caused by other agents used in conjugation.

Table 16. Preparation of Salmonella Ab and GNPs conjugate with negative controls, and their UV-Vis Spectra before and after centrifugation

1	**2**	**3**	**4**
10 ml GNP	10 ml GNP 1 ml %10 BSA in 20 mM sod.borate	10 ml GNP 2ml PBS 1,2ml %10 BSA in 20 mM sod.borate	10 ml GNP 2ml **Ab** in PBS 1,2ml %10 BSA in 20 mM sod.borate
Resuspended in WB	Resuspended in WB	Resuspended in WB	Resuspended in WB

UV-Vis	**1**	**2**	**3**	**4**
Before centrifugation	526nm	526nm	526nm	526nm
After centrifugation	527nm	527nm	528nm	532nm

Conjugation of GNPs with *E.coli* Ab was achieved according to the method 2 (Guo et al., 2009) with slight changes. Briefly, 2 ml of dialysed Ab with desired concentration, 35 µg/ml, was swiftly added to 10 ml of GNPs (pH: 8.0). Then it was allowed to incubate for 1 hr before stabilizing the sol with 1% BSA and 0.01% PEG as final concentrations. After 5 min stirring, the mixture was incubated for 1 hr and then centrifuged at 20000 RCF for 30 min at 4˚C. Supernatant was carefully discarded and pellet was resuspended with 10 ml of 0.01 M PBS buffer (pH: 8.0) containing 1% BSA and 0.01% PEG8000 and then allowed to centrifuge at the same conditions as before twice. Finally, Ab/GNPs complex was dissolved in 1 ml PBS buffer (pH: 8.0) involving 10% sucrose, 1% BSA, 0.05% sodium azide which is called as resuspension buffer (RB), and stored at 4˚C for the next use. Table 17 demonstrates the prepared GNPs with/without Ab.

Table 17. Preparation of *E.coli* Ab and GNPs conjugate with negative controls and their UV-Vis spectra before and after centrifugation

1	**2**	**3**
10 ml GNP	10 ml GNP 2 ml PBS 1% BSA 0.01% PEG8000	10 ml GNP 2 ml Ab 1% BSA 0.01% PEG8000
Resuspended in RB	Resuspended in RB	Resuspended in RB

UV-Vis	**1**	**2**	**3**
Before centrifugation	526nm	527nm	527nm
After centrifugation	528nm	530nm	533nm

2.3.3.2. Designing of Lateral Flow Strips

Assemblying of the strip components was made by overcrossing the pads on NC membrane with 2 mm overlapping. While the conjugate and sample pads were placed at the beginning of the membrane, absorbent pad was affixed to the distal end (Figure 13). The reagents on capture zones were manually applied in 0.6 µl PBS with desired

concentrations as 4-5 droplets. Table 18 also shows the buffers used for designing of LF strips.

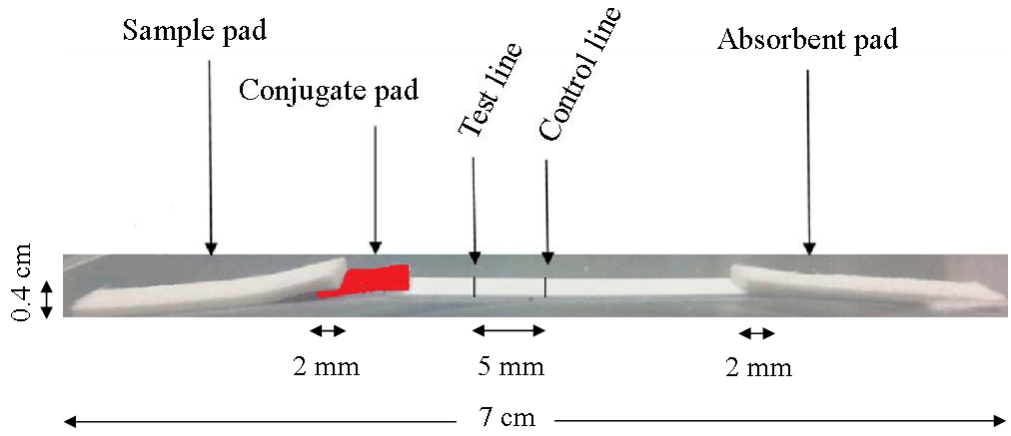


Figure 13. Assemblying of the strip components onto backing material

Table 18. Buffers used for developed lateral flow strips

	Conjugate pad buffer		**Sample pad buffer**
20mM sodium borate (pH:8.0) containing**;**	2% (w/v) BSA 3% (w/v) sucrose 0.6 M NaCl 0.2% (v/v) Tween 20 0.1% (w/v) sodium azide	1X PBS (pH:7.2) containing;	0.1 M NaCl 0.2% (v/v) Tween 20 0.1% (w/v) sodium azide

2.3.3.2.1. Single Chain Variable Fragments based Strips

Initially, 0.6 µg/strip LPS was immobilized on NC membrane to make sure that conjugate is able to capture the antigen on the strip. Then 0.6 µg/strip antigoat IgG and 1.4 µg/strip scFv in PBS were dispensed in the form of droplets onto NC membrane as a test and control line respectively. Then, it was dried at 37˚C for 15 min. Besides, LPS and IgG was also experienced as a test and control line or vice versa to see the capturing efficiency of the location of the agents. Conjugate pads were prepared by cutting fiberglass to 3.5 cm, and 50 µl of scFv-GNPs conjugate was diluted with 100 µl of conjugate pad buffer. Then, it was applied to the pad and allowed to dry at 37˚C

for 1 hr. Sample pad was cut to 7 cm and dried at 37˚C for 1 hr after soaking with sample pad buffer. All the prepared membranes were stored at dry conditions. The treatment of samples on to the designed strips was performed by either loading the 100 µl of sample in PBS on the sample pad or dipping the strips into the eppendorf tubes including 200 µl fresh cultured sample which is measured as 0.5 at OD_{600}. The strips were washed with 50 µl PBS once if desired.

2.3.3.2.2. Common Structural Antigenic Antibodies based Strips

The strip design, buffers used for developing LF strip and sample treatment were the same as described above with slight differences. Briefly, desired concentration of either Salmonella or *E.coli* O157:H7 Ab and affinity purified antibody to goat IgG was affixed on to NC membrane as a test and control line, respectively.

2.3.3.3. Optimization parameters of developed Lateral Flow Strips

2.3.3.3.1. Antibody concentrations on the capture zones

Stock concentrations of Salmonella Ab, *E.coli* O157:H7 Ab and affinity purified Ab to goat IgG were prepared in PBS. Then 0.6 µl/strip Abs with desired concentrations was spotted on the capture zones.

2.3.3.3.2. Conjugate solution on the conjugate pad

Conjugate pad was prepared by applying the mixture of 100 µl Ab/GNPs conjugate and 200 µl conjugate pad buffer on to 7 cm fiberglass membrane for both Salmonella and *E.coli* O157:H7 test strips then called as conjugate A and A1, respectively. To make a comparison of either concentrated or diluted conjugate solutions on the pad, various mixtures were prepared. Briefly, 5 and 2.5 times concentrated conjugates were loaded on the pad for Salmonella and called as conjugate B and C, respectively. Conjugate D was prepared by diluting the conjugate A with a ratio of ¼ before loading for Salmonella detection. In terms of *E.coli* strips, 2 fold serially diluted conjugate A1 was loaded on the conjugate pad and called as conjugate B1 and C1. Conjugates were also prepared by 5, 6, 2 and 8 months old GNPs for Salmonella stips and called as

conjugate 1, conjugate 2, conjugate 3 and conjugate 4, respectively to see the stability of GNPs and bioconjugates.

2.3.3.3.3. Comparison of the membrane types

HF240MC100, HF180MC100, HF120MC100 and HF075MC100 NC membranes were experienced to find the best membrane type to develop the rapid and efficient test platform without giving any background signal.

2.3.3.3.4. Limit of detection (LOD)

LOD of target bacteria was determined using both heat inactivated Salmonella, $5x10^9$ cells/ml, and *E.coli* O157:H7, $3x10^9$ cfu/ml, cells by making 10 fold serial dilutions in PBS. 100 µl of sample volume was applied to the sample pad and 50 µl PBS was added for washing the strip in a 5 min if desired.

2.3.4. ELISA

2.3.4.1. Determination of the antigen binding activity of Single Chain Variable Fragments

An indirect ELISA was performed to analyze the antigen binding activity of purified scFv. Initially, colony forming unit of *S. typhimurium* 14028s and LT2 strain was determined by plate counting method as triplicate after making 10 fold serial dilutions. Formalin killing (FK) procedure for the cells was also applied as follows. Briefly, 15 ml of fresh culture which OD_{600} value is 0.5 was centrifuged at 4255 g for 20 min at 4˚C and resuspended in 10 ml PBS containing 0.3% formaldehyde after washing with PBS for three times, and allowed to incubate for 24 hr at RT. On the next day, it was pelleted at the same conditions and resuspended in 1 ml PBS after washing with 10 ml PBS twice. In terms of the FK 14028s cells, procedure was applied as the same way mentioned above except for the initial culture volume which is 150 ml. Then both strains were stored at -20˚C until use. A 96 well polystyrene microtitration plate was used for the binding assay. For the purpose of testing the affinity of scFv to the serogroup D LPS, *S.typhi* LPS coated microtiter plate provided by ELISA kit was used.

Salmonella LPS, live or FK Salmonella cells were used to coat the plate for testing the binding efficiency of scFv to the serogroup B. $7.3x10^6$ and $2.8x10^7$ cells for 14028s and LT2, respectively were for the live cell coating, while $1.5x10^7$ and $4.2x10^6$ cells for 14028s and LT2, respectively were for the FK cells coating per well in 100 µl coating buffer (CB: 0.05M Sodium carbonate, pH: 9.6). Microtiter plates were incubated overnight at 4˚C. After washing the wells three times using 200 µl PBST (0.1% Tween 20), the plates were blocked for 4 hr at 37˚C with 3% skim milk powder in PBS. 100 µl of purified scFv Ab was diluted by making 1/3 serial dilutions with desired concentrations in PBS and run in duplicate to the antigen coated wells before incubating for 1 hr at RT with gentle shaking. After washing, the plates were reincubated for 1 hr on a rotating shaker at RT with 100 µl/well of anti-His secondary Ab which is diluted as 1:2000 in PBST. After washing step, the enzymatic reaction was developed with adding 50 µl of TMB substrate and reaction was stopped by adding 50 µl of 1 M H_2SO_4 in each well. The optical density on plates was measured by use of microtiter plate reader at 450 nm dual wavelength. Both $2.5x10^7$ cfu/ml *E.coli* cells and CB were used for coating the wells as negative controls. The overnight cultures of *S. bongori*, *S. ohio* and *S. newport* were also used to test the binding ability of purified scFv to the different *Salmonella* serogroups after coating the microtiter plate. K_D values of scFv to the targets were calculated by SigmaPlot 10.0 version using absorbance values.

2.3.4.2. Detection of *S. typhimurium* in the bacterial mixture

In order to test the binding capability of produced scFv to the target bacteria when it is included in the mixture, different ratios of bacterial mixtures were prepared. Briefly, desired cell numbers in 100 µl CB was mixed after OD_{600} value is reached to 0.5 and applied to the wells as 200 µl total test volume. The ratios of bacterial mixtures were adjusted as 10^7:10^7, 10^6:10^7, 10^5:10^7, and 10^4:10^7 cfu/ml for the *S. typhimurium* LT2 and *E.coli*, respectively for coating the wells. All steps for the ELISA were applied as the same with above.

2.3.4.3. Detection of *S. typhimurium* using fusion antibody

Stuff supplied in Salmonella ELISA Kit was used to test the binding efficiency of fusion Ab to the serogroup D LPS and serogroup B antigens by applying the procedure given in manual. FK *S. typhimurium* LT2 and *S.typhimurium* LPS was used for coating the 96 wells plate in order to see the affinity of fusion Ab to the serogroup B. Briefly, 5 µg/ml LPS and $4.2x10^8$ *S. typhimurium* LT2 cells in 100 µl 0.05 M carbonate buffer was used for coating the wells and incubated at 4˚C for overnight. After washing the wells with wash buffer for three times, they were blocked with 200 µl of 3% skim milk powder in PBS at 4˚C for 4 hr. Then washing step was repeated before adding the fusion Ab to the 1st wells with desired concentrations in 100 µl sample diluent and serial Ab dilutions were done. After 30 min incubation time at RT, procedure provided by Kit manual was applied to the assay. For the negative controls, wells were coated with sample diluent without including any antigen and also coated with *E.coli* cells using overnight culture after washing them with PBS twice. Additionally, Salmonella Abs and S. *typhimurium* LPS factor 4 Abs were also added into the serogroup B antigens, seperately to make a comparison between the humanized fusion Ab and commercial Abs specific to Salmonella antigens or O factors.

2.3.4.4. Immunomagnetic based ELISA

2.3.4.4.1. Conjugation of fusion antibody with magnetic beads

Two types of magnetic beads which are uniform (Promag) and nonuniformed sized SNAP capture magnetic beads (NEB) were used for coupling of fusion Ab. To couple of the Ab with Promag beads, in a diameter of 0.8 µm and containing COOH, beads linked with BG substrate were obtained from the Ellington lab. After removing the excess substrate via magnetic separation, 20 µl of fusion Ab comes from 1.6 mg/ml stock and 50 µl of BG-coupled beads were mixed and allowed to rotate at 4˚C for overnight. On the next day, the coupled Ab with beads was washed with 100 µl PBST (0.1% Tween 20) twice via magnetic separation and called as couple 1. Moreover, 25 µl of fusion Ab comes from 4,4 mg/ml stock and 80 µl of BG-coupled beads were also mixtured. The coupling reaction was performed by the same procedure as described

above and called as couple 2. This was used to see the efficiency of the amount of Ab and beads for capturing assay. Non-coupled magnetic beads with BG was also mixed with Ab and used as negative control. The SNAP capture magnetic beads, 75-150 µm, which have already BG substrate (Figure 14) were washed with PBS once before coupling with fusion Ab. Then 20 µl of Ab comes from 0.63 mg/ml stock and 80 µl of beads were mixtured and allowed to rotate at 4˚C for overnight. After washing step as mentioned above, 2 µl of Ab coupled with magnetic beads were used for ELISA and called as couple 3.

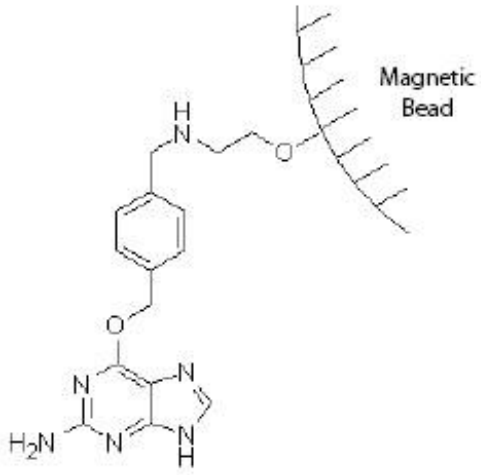


Figure 14. The SNAP-Capture Magnetic Beads coupled with BG substrate (NEB)

2.3.4.4.2. Magnetic capturing of *S. typhimurium* by fusion antibody

Both the cultures of 14028s and *E.coli* cells were used after their OD_{600} value reaches to 0.5 and capturing assay was performed as duplicate. Briefly, 5 ml cultures were grown at 37˚C and 1 ml of cells were pelleted at 10000 xg for 4 min and then washed with PBS twice. After resuspending of pellet in 1 ml PBS, 10 fold serial dilutions were done by taking 10 µl of culture and adding it into 90 µl PBS. For magnetic capturing assay using Promag beads, both $7.3x10^2$ cfu/ml *S.typhimurium* 14028s in 100 µl and $2.5x10^3$ cfu/ml *E.coli* in 100 µl were divided into 20 µl aliquots. Then they were incubated with pre-prepared Ab bead complexes at RT for 1 hr as rotating. After washing with 100 µl PBST (0.1% Tween 20) for four times via magnetic separation,

20 µl of enzyme conjugate obtained from ELISA Kit was added and allowed to rotate at RT for 1 hr. Then, 100 µl of TMB solution was added into the mixture after the same washing step. Following the 10 min incubation time, reaction was ended with 100 µl of 1 M sulphiric acid addition. The optical density was measured by the use of microtiter plate reader at 450 nm after separating magnetic beads from the mixture.

The magnetic capturing of *S. typhimurium* 14028s using NEB beads was proceeded as the same way described above with slight changes. Assay samples were prepared as triplicate. $2.5x10^4$ cfu/ml *E.coli* in 100 µl was prepared and 20 µl of aliquots was used as a negative control and 50 µl of PBST was used for washing the capturing assay for five times.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Bacterial growth

S. typhimurium 14028s& LT2 and *E.coli* were grown in LB and their colony forming units were calculated as $7.3x10^8$ cfu/ml, $2.8x10^8$ cfu/ml and $2.5x10^8$ cfu/ml, respectively after plate counting method. They are used for all experiments after their OD_{600} value reaches to 0.5. FK 14028s cells were prepared as $1.1x10^{11}$ cfu/ml and $4.2x10^9$ cfu/ml was for LT2 stock solutions. Either overnight grown cultures or freshly prepared cultures of other bacteria were used for strip assays.

3.2. Generation of recombinant antibodies

Before adopting to the test platforms, scFv and humanized fusion Ab form of se155-4 Ab specific to Salmonella were engineered, expressed and purified succesfully.

3.2.1. Production of Single Chain Variable Fragments

Bacterial expression of scFv was achieved and purified in a high concentration after constructing pET-28a (+) vector with synhetic gene block of scFv. Successful transformation, expression and purification results were described in the following sections.

3.2.1.1. Transformation to *E.coli* cells

As a beginnig, vector PCR and scFv gene block PCR were performed at the Ellington Lab, separetly. After verification on the agarose gel electrophoresis, gel extraction of DNA pieces was done. Gibson assembly reaction was achieved using extracted DNA

pieces in order to insert the scFv genes into plasmid DNA. Then, scFv inserted plasmid DNA was transformed to *E.coli* cells and then allowed to grow on agar plate for overnight. After transferring of single colony into the 5 ml LB it was incubated for overnight. On the next day, miniprep was applied to the liquid cultures of transformed cells to isolate the plasmid DNA. DNA concentration was quantified as 50.5 ng/µl and further verification was performed by PCR in order to see the insertion of scFv.

3.2.1.2. Isolated Plasmid PCR

PCR results of scFv gene block inserted in plasmid DNA and gel image of isolated whole plasmid DNA were shown in Figure 15. As it is seen in the image, scFv genes were efficiently inserted into plasmid DNA and PCR result verified it with the expected size, i.e., 871 bp (A). Whole plasmid DNA without containing scFv genes was also demonstrated on the agarose gel image, ~5200 bp, (B) and 1 kb DNA ladder was used for verifications on the gel image.

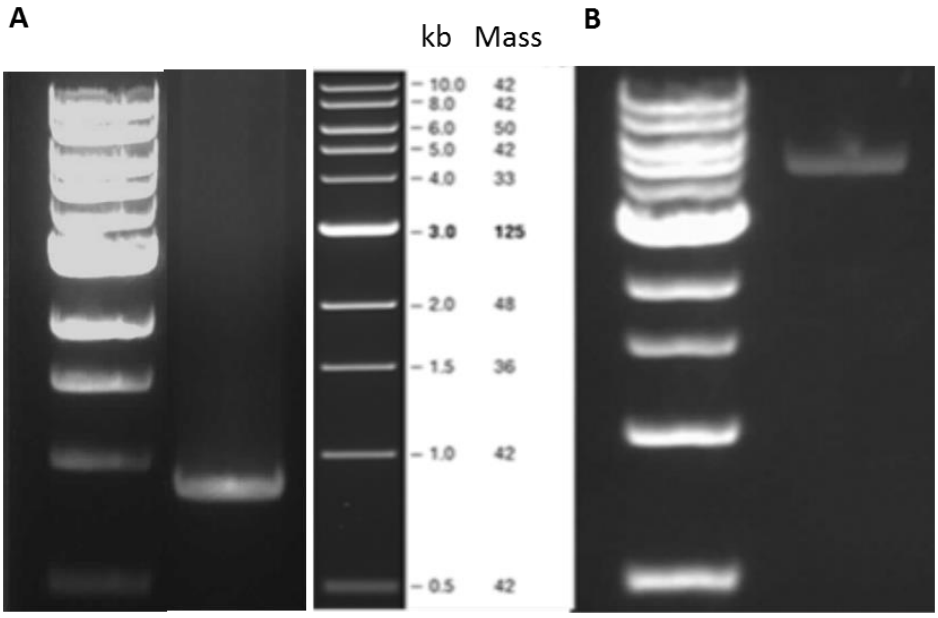


Figure 15. Agarose gel image of PCR product of scFv gene block in plasmid DNA **(A**) and whole plasmid DNA without containing scFv **(B)**

3.2.1.3. Expression of Single Chain Variable Fragments

E.coli cells were further transformed with scFv inserted plasmid DNA firstly, and transformed cells were obtained by growing the cells on LB agar containing Kan. On the next day, single colony was transferred into 2XYT media, and 10 ml of overnight cultures as a total volume was added in the expression media, superior broth. Figure 16 depicts the transformed cells with scFv inserted plasmid on the agar plate containing Kan, and pre-inoculation of the single colony into 2XYT media.

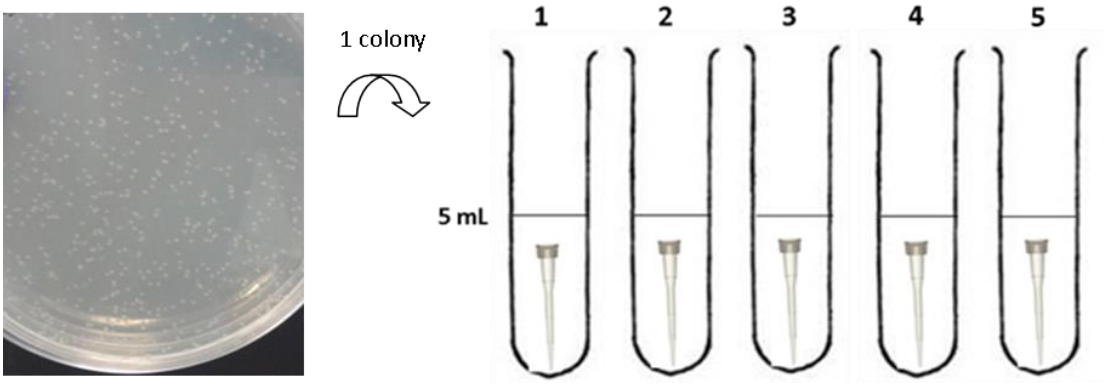


Figure 16. Transformed cells with scFv inserted plasmid on the Kan added agar plate and pre-inoculation of single colonies into 2XYT media

It was reported that the yield of produced scFv is proportinal to the induction times. 6 h was found as optimal time for formation of the max scFv yield and longer incubation may be resulted with lower Ab production (Brichta et al., 2003). However, results showed that 24 h induction seems to enough time for scFv expression in a high concentration in this study.

3.2.1.4. Purification of Single Chain Variable Fragments

Expression of scFv was succeded and Ab product comes from periplasmic fractions after purifying by metal affinity chromatography was obtained purely. As it is seen in the Figure 17, FT, W1 and W2 samples contain contaminating protein while scFv proteins do not, after column purification (A). Further FPLC purification was also

applied to increase the purity (B). Batch to batch expression studies showed that produced scFv concentrations were between 2.5 and 3.5 mg/ml in final 500 µl volume. As expected the size of the purified peptide was aroud 27 kDa.

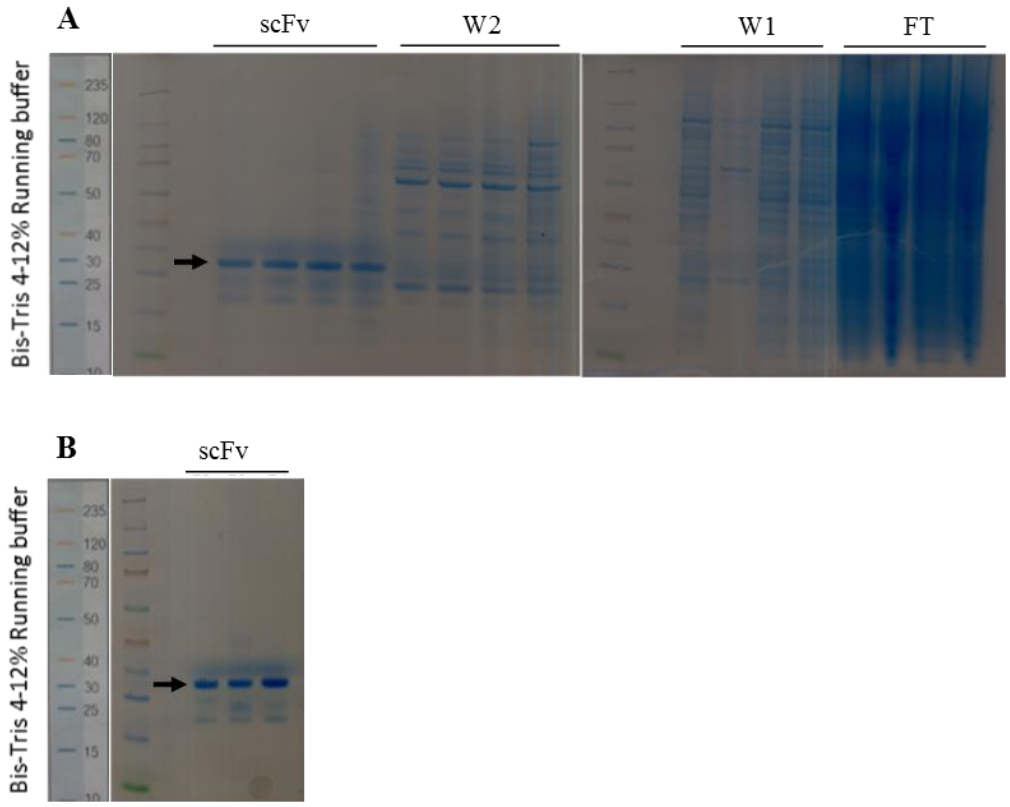


Figure 17. SDS PAGE gel image of column **(A)** and FPLC **(B)** purified scFv antibody. W2: Wash 2, W1: Wash 1, FT: Flow Through. Arrows indicate purified scFv

Since the elevated temperature might be resulted with reduced binding [Krauss et al., 2004], the storage temperature of scFvs has a significant role for binding affinity to their antigens. In this study, after 9 months anti Salmonella scFv was found to have considerably good stability at -80˚C without adding any stabilizers or protease inhibitors. However, some scFvs were shown to lost stability at -80˚C in storage buffer containing protease inhibitors and gylcerol (Brichta et al., 2003). Although Tris-HCl, NaCl (Pleckaityte et al., 2011) and 0.01% sodium azide (Karsunke et al., 2012) may be helpful for long term scFv storage at 4˚C, generated scFv in PBS without any supplement was found in a good stability at 4˚C for 7 days in this work. Because

keeping at 4˚C as stable is also possible without adding protective (Pleckaityte et al., 2011, Jäger et al., 2013).

3.2.2. Production of fusion antibody

pSNAPf vectors were designed for the construction of both heavy and SNAP-Tagged light chains of se155-4 Ab, separately in the Ellington lab (Figure 18, 19).

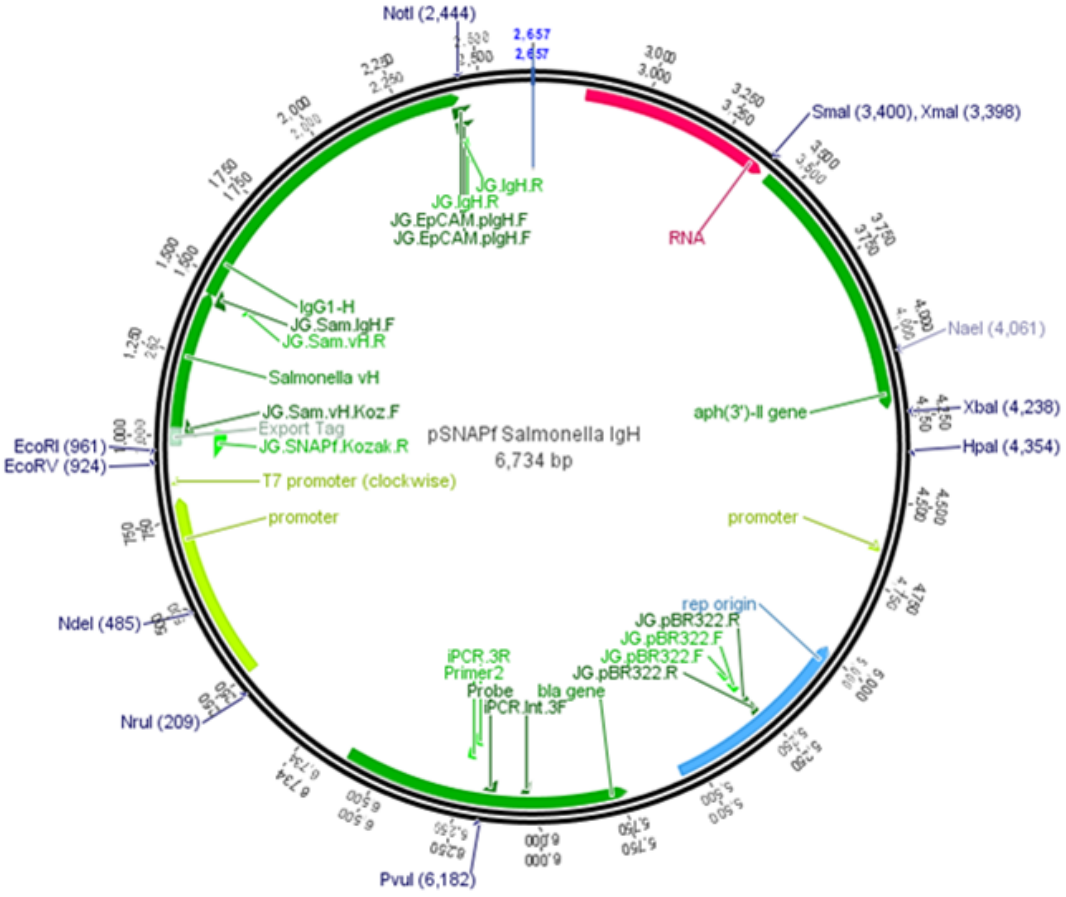


Figure 18. Construction of pSNAPf vectors for the heavy chains (IgH) of full length fusion antibody

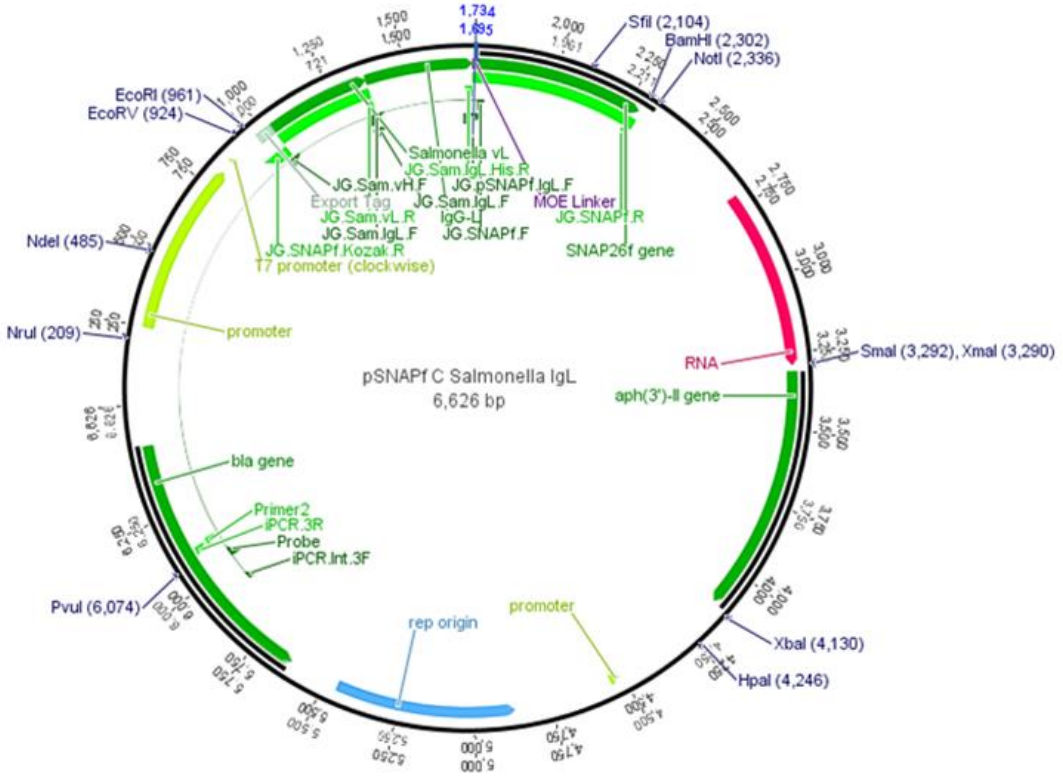


Figure 19. Construction of pSNAPf vectors for the SNAP-Tagged light chains (IgL) of full length fusion antibody

After transformation procedure, concentrations of isolated plasmids inserted with the heavy and SNAP-Tagged light chain sequences were 1092 ng/µl and 1200 ng/µl, respectively.

3.2.2.1. Transfection and expression of fusion antibody

Transfection of Expi293 cells with plasmids having Ab chains was achieved. Expression of fusion Ab in mammalian cells was lasted about 6 days in 37˚C incubator with a humidified atmosphere of 8% CO_2 and shaking at 125 rpm.

3.2.2.2. Purification of fusion antibody

Column purified fusion Ab ~55 kDa was shown in Figure 20. Although it was obtained purely, further FPLC purification was performed and used for both indirect color based

normal ELISA and immunomagnetic ELISA. The concentrations of fusion Ab purified from HEK293 cells were found as 0.63, 1.6 and 4.4 mg/ml in batch to batch expression dependently on the number of used culture flasks. This is considerably high concentration compared to the 0.01 mg/ml scFv-Fc protein expressed in HEK293T cells (Jäger et al., 2013). This result also demonstrated that generated fusion Ab has high expression profile in mammalian cells.

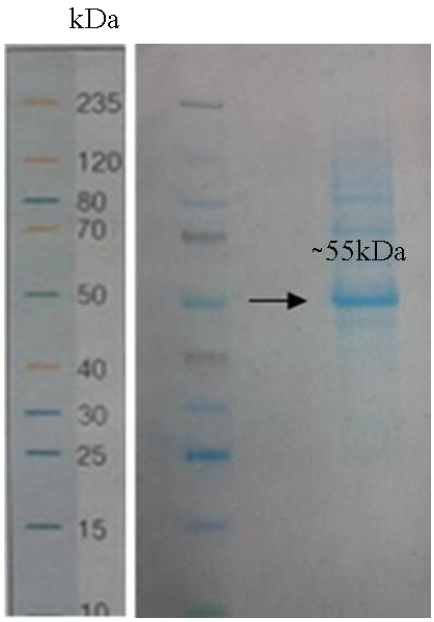


Figure 20. SDS PAGE gel image of column purified fusion antibody

3.3. Development of Lateral Flow Strip Assay

3.3.1. Synthesis of Gold Nanoparticles and conjugation with antibodies

The most popular method for the synthesis of GNPs based on the reduction of gold derivatives for a long time is reduction of $HAuCl_4$ by citrate in water which was introduced in 1951 by Turkevitch (Turkevitch et al., 1951, Daniel and Astruc, 2004, Kimling et al., 2006). In this method, reducing and stabilizing agent is citric acid and it provides GNPs with various diameters by adjusting the ratio of gold salt to citrate (Frens, 1973). Therefore, particle size may be highly precised with the known initial

concentration of gold which is used for the synthesis of nanoparticles (Haiss et al., 2007). In this study, different sized GNPs were synthesized according to the citrate reduction method and filter sterilized before using. Two different methods, i.e., method 1 and method 2 were used to couple of the Abs with GNPs, and both were found as having a good stability for long term usage which is about 8 months at 4˚C.

3.3.1.1. Synthesis of 16 nm GNPs and conjugation with Single Chain Variable Fragments

Using the appropriate amount of citrate molecules, 16 nm sized GNPs which have the max absorption (λ_{max}) at 518 nm wavelength were synthesized with a homogenous distribution and used for coupling with scFv. In the labelling of GNPs process, the pH of coupling mixture plays significant role. Because ionic bonds can be occured between the GNPs which have negative charges over a wide range of pH and a partially protonated protein which is at suitable pH. Figure 21 A demonstrates the stability (λ_{max}/580 nm) and polydispersity (600 nm/λ_{max}) of GNPs/scFv conjugate at different pH. While the color intensity of conjugate seen at pH: 8.5 - 9 and 9.5 seems similar (A), optimum pH value was determined as pH: 9 after adding 40 µg/ml scFv to the each well since the conjugate seems to stable and least polydispersed at pH: 9. This is expected because minimum electrostatic interactions and maximum Ab adsorption are occured on GNPs at this pH via hydrophobic interaction with the Ab (Norde, 1986, Oliver, 1999). Higher pH value is also needed for the smaller GNPs to coat them with Ab compared to the larger nanoparticles (Lou et al., 2012). As expected pH values below pH: 7.5 also caused nanoparticle aggregation and resulted with grey color after adding high salt (A).

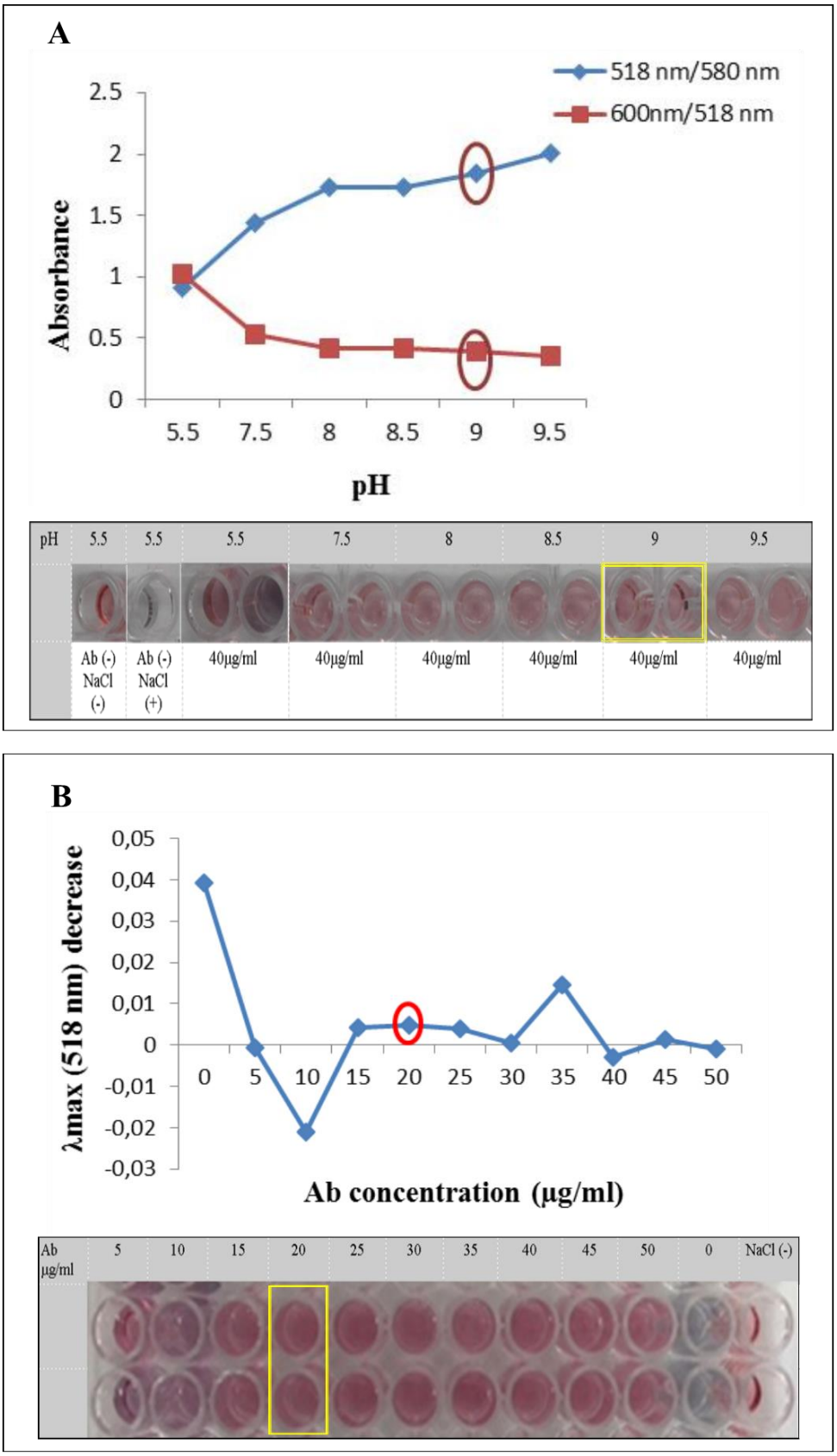


Figure 21. Optimum pH value **(A)** and scFv antibody concentration **(B)** for coating GNPs

The changes in SPR of GNPs were assessed for conjugation process to choose the minimal amount of Ab needed for protecting the GNPs from salt induced precipitation after exposure to high salt. Therefore, absorbance decrease at λ_{max} obtained from spectrophotometric analysis was measured to make precise evaluation of the GNPs stabilization (Horisberger and Rosset, 1977). Then the optimum amount of scFv was choosen based on the minimum decrease in absorbance at λ_{max} (Figure 21 B). As it is seen in the graph, the decrement of absorbance was reduced at the concentrations of 15 µg/ml. When the concentration of Ab exceeded this amount the curve became flat approximately. Thus, the minimum scFv Ab was selected from the point where the curve appeared to be relatively parallel to the X axis in the graph. Therefore, the optimal amount of Ab was decided as 20 µg/ml of scFv, which was resulted by the same red color intensity with naked GNPs without any aggregation (B) after labelling process. Although 15 µg/ml seemed as minimal amount for conjugation, a slightly more concentration was chosen. Because decided Ab concentration should be greater than the minimum amount of Ab concentration to make a good stabilization of GNPs (Shi et al., 2008). Even if there is a study reporting that 4 µg/ml is the minimum Ab concentration to avoid agglomeration of 16 nm sized GNPs, it was less sensitive to detect the real sample (Lou et al., 2012). Besides, 150 µg/ml Ab was given as the best amount for labelling of 20 nm sized GNPs (Mikawa et al., 2009), means that more Ab is needed for coating. However, it was reported that the Ab concentration used might be low if the nanoparticle size is 13 nm (Ye, 2010). Adding a low amount of Ab to the GNPs also caused particle flocculation characterised by a blue grey colour. This is resulted by reduction of electrostatic repulsive charges occurred at between the particles in the presence of high concentration of electrolyte. After determining the optimal pH and Ab concentration, flocculation had not been observed with the addition of high salt. This means that optimum amount of adsorbed scFvs can confer stability to the GNPs by preventing aggregation (Chun, 2009). Interestingly, when the amount of examined Ab exceeded 30 µg/ml, unexpected issue was occurred in the graph while it was found as normal red color for the conjugates in the wells. It was reported that steric hindrance, occuring of multiple protein layers and Ab orientation may lead poor conjugate resulted by excess amount of Ab (Thobhani et al., 2010). This reason may be one explanation of this unusual behaviour.

The conjugation of GNPs with scFv could be performed by adding histidine or cystein residue into the scFv linker (Liu et al., 2009), scFv framework region or the end of C terminal (Ackerson et al., 2006) for directly coupling. However, GNPs were labelled with scFv via non-covalent interaction and it showed good immobilization of scFv on GNPs in this study. scFv stabilized GNPs retained the bright red color and they were found as stable for 3 weeks in the coating buffer at 4˚C. This result is in parallel with the study showed that either 30 nm or 50 nm sized GNPs had shown the least stability compared to 15 nm sized GNPs labelled with scFv (Liu et al., 2009). Figure 22 displays the UV-Vis and size measurement of both naked and scFv labelled GNPs. As it is seen, the maximum absorbance of naked GNPs shifted from 518 nm to 528 nm after coated with scFv (D). This result is expected because optical properties of GNPs can be changed by the size increment, shape or adsorption of proteins on GNPs (Jain et al., 2006). About 6-10 nm shifted absorbance of GNPs capped with Ab is also expected (Nghiem et al., 2010). The centrifugal steps and adding of BSA into the GNPs solution was resulted with a slight shifting in the λ_{max} (B, C). To say that BSA adsorption might be independent of GNPs size (Dominguez-Medina e tal., 2012). The average diameter of synthesized GNPs was measured as 16 nm (E) while 93 nm was for the conjugate (F). This shows the evidence that scFvs were loaded onto GNPs, succesfully.

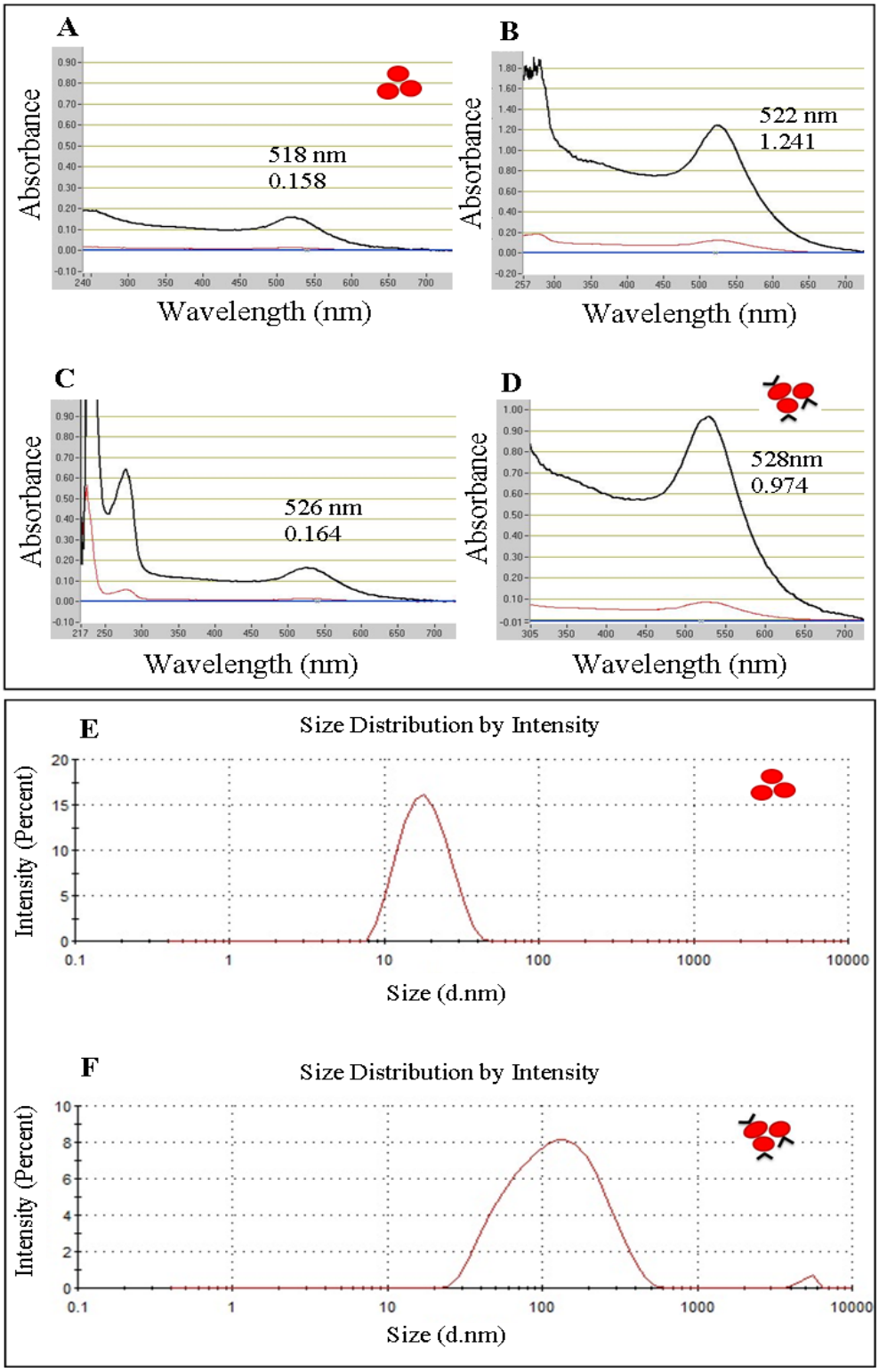


Figure 22. UV-Vis spectra **(A, B, C, D)** and size distribution of synthesized GNPs and GNPs/scFv conjugate measured by Malvern Zeta Sizer instrument **(E, F). A)** Dialyzed naked GNPs **B)** GNPs/BSA after centrifugation **C)** GNPs/BSA/scFv before centrifugation **D)** GNPs/BSA/scFv after centrifugation **E)** Dialyzed naked GNPs **F)** GNPs/scFv conjugate

Figure 23 also displays the scFv alone and GNPs/scFv conjugate on the SDS agarose gel electrophoresis. It is clearly seen that all Abs used were adsorbed on the surface of GNPs. The band sizes of GNPs/scFv conjugate (2-3) and GNPs/BSA (5) are seen as almost the same because BSA was already used for stabilizing the GNPs along with the scFvs. Using 5 times concentrated GNPs called as 5X in deeply red color and naked GNPs called as 1X (a, b) were not seen on the agarose gel without protein coupling (4, 6).

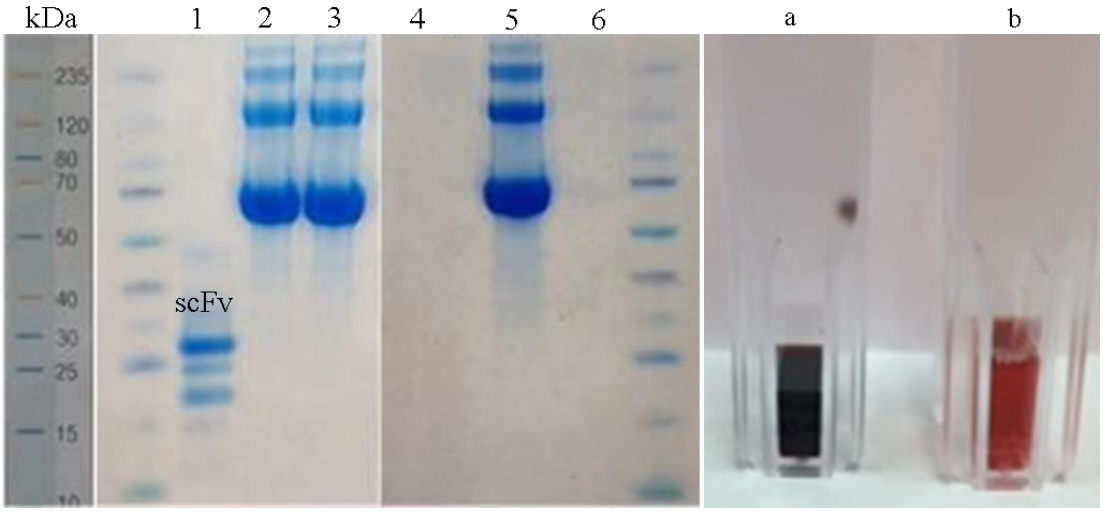


Figure 23. SDS PAGE image of scFv, naked GNPs and GNPs/scFv conjugate, and image of 1X and 5X GNPs. **1)** scFv **2)** 1X GNPs/scFv conjugate **3)** 5X GNPs/scFv conjugate **4)** 5X GNPs **5)** 1X GNPs/BSA **6)** 1X GNPs. Ladder: Multicolor broad range protein ladder **a)** 5X GNPs **b)** 1X GNPs

3.3.1.2. Synthesis of 36 nm GNPs and conjugation with Common Structural Antigenic Antibodies

36 nm GNPs were synthesized by addition of the appropriate amount of sodium citrate since it yields certain GNPs sizes (Oberdorster et al., 2004, Qu and Lü, 2009). In a comparison, the plasmon scattering of dyes or quantum dots is 10-100 times weaker than of the 36 nm sized GNPs (Saha et al., 2012). Synthesized GNPs were demonstrated in Figure 24. Although it is difficult to obtain the reproducibility in regards to size distribution from batch to batch preparation of GNPs, they were obtained homogenously and well distributed spherical nanoparticles. In the solution,

the stabilization of GNPs is possible by adsorbed negative ions such as citrate. As the Van der Waals attraction between the GNPs are also perevented by its repulsion, no aggregation is occured (Hunter, 2001, Shaw, 1991). The λ_{max} of synthesized GNPs was recorded at 526 nm or 524 nm wavelength as expected and sterile distilled water was used as blank for UV measurement. The concentration of GNPs was calculated as $1.18x10^{14}$ particles in 0.19 nM since it is possible to calculate the extinction coefficient of different sized and shaped GNPs, theoratically (Jain et al., 2006).

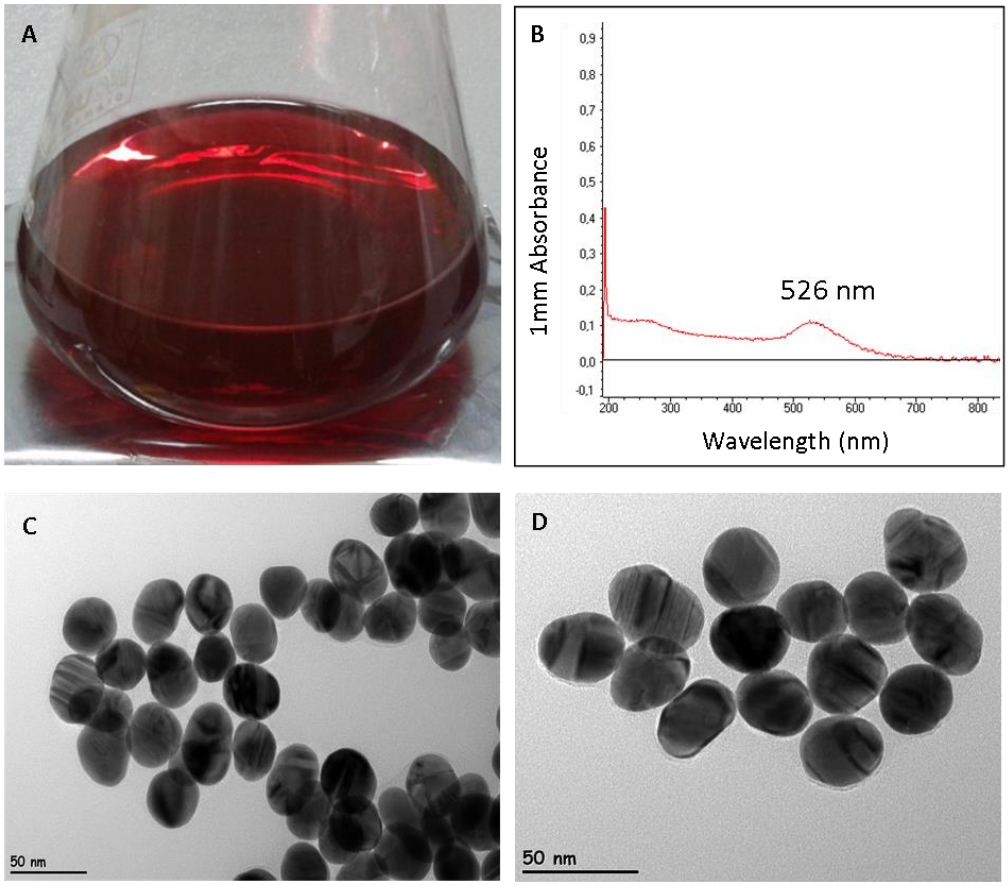


Figure 24. Synthesized 36 nm GNPs **A)** Colloidal naked GNPs **B)** UV-Vis spectrum of naked GNPs **C, D)** TEM images of naked GNPs. Scale bar: 50 nm

pH value has significant role for the proteins and their conjugation process. For instance, IgG is assumed as "T" form at pH: 10 or 4 and it occupies its max area. However, the molecule might have a "Y" form at pH values between either pH: 4 or pH 10 and the point of zero charge (Geoghegan, 1988). The stability and dispersity of

colloidal GNPs are linearly and directly related with the ratio of absorbancy at λ_{max} : 580 nm and 600 nm : λ_{max}, respectively. To say that, recording high absorbance at 580 nm is caused by particle aggregation and it gives rise to destabilization of gold sol, while the higher value of the ratio of 600 nm and λ_{max} means the more polydisperse gold sol. Figure 25 A and Figure 26 A demonstrates the stability and polydispersity of GNPs conjugated with Salmonella and *E.coli* O157:H7 Ab, respectively at different pH after salt addition. It was found that GNPs conjugates prepared for the Salmonella and *E.coli* detection are least polydisperse and most stable at pH: 9 and pH: 8, respectively. This result is expected because to make a conjugation of GNPs in the range of 5-60 nm with Ab is generally possible at the pH values between 8.00 and 9.5 (Mikawa et al., 2009, Safenkova et al., 2010). Besides, complex is more stable with higher pH value (Ao et al., 2006) even if one study showed that lower pH value is also suitable for conjugation of different sized GNPs (Lou et al., 2012). It is clearly seen that adding of NaCl caused the aggregation of nanoparticles resulted with purplish color when the pH is lower than 8.00 even if the Ab concentration is high in the solution. Figure 25 B and Figure 26 B depicts the λ_{max} decrease versus the increasing amount of Salmonella and *E.coli* Ab, respectively. Increased amount of Ab enhanced the stability of GNPs, and it became constant after the concentration of 12 µg/ml Ab for Salmonella and 15 µg/ml Ab for *E.coli*. However, 20 µg/ml of Salmonella Ab and 35 µg/ml of *E.coli* Ab was decided to coat the GNPs since the red color intensity of conjugates is the same with naked GNPs and no agglomeration was occurred after adding NaCl into the solution. This result showed similarity with the studies reported by Ang et al. (Ang et al., 2012) and Zhao et al. (Zhao et al., 2010) which they used about 22 µg/ml and 30 µg/ml Ab to coat the 40 nm sized GNPs. However, it was comparable with the study reported that optimal amount of the anti *E.coli* O157:H7 Ab was 12.0 µg/ml for 40 nm sized GNPs (Suria et al., 2015). While the minimum Ab required for coating the GNPs sized with about 40 nm were generally reported as high concentrations such as 150 µg/ml or 40 µg/ml in the literature (Mikawa et al., 2009, , Tripathi et al., 2012, Chenggang et al., 2008), 12-16-20-35 µg/ml were found as ideal concentration for 36 nm GNPs in this study. Although the binding capacity of IgG Ab to per GNPs was not quantified in this work, it was reported that 100-150 IgG Abs were coated on a single 36 nm GNPs (O'Farrell, 2013).

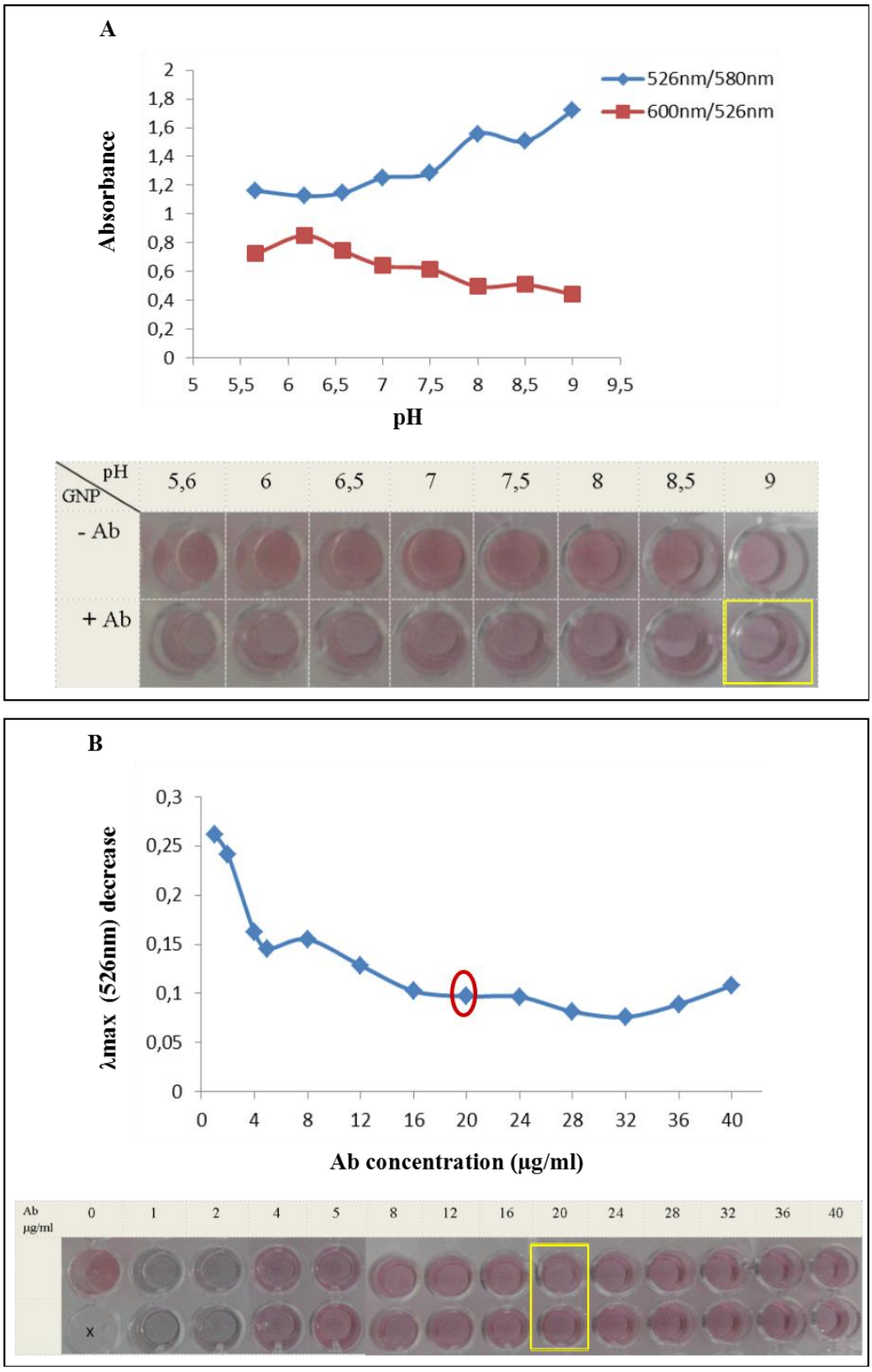


Figure 25. Optimal pH value **(A)** and Salmonella Ab concentration **(B)** for coating GNPs

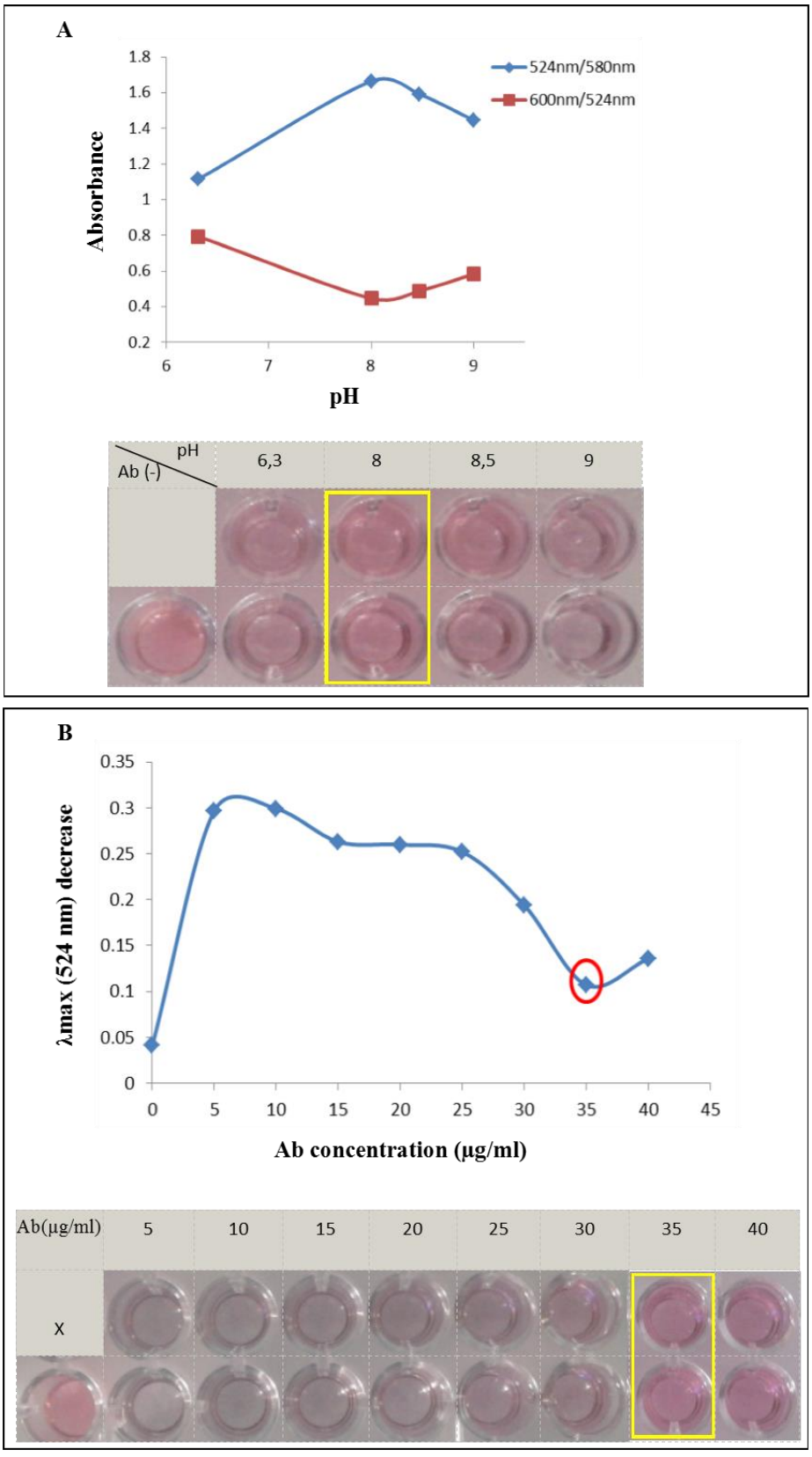


Figure 26. Optimal pH value **(A)** and *E.coli* 0157:H7 Ab concentration **(B)** for coating GNPs

It was found that batch to batch sample preparation results were consistent. This also indicates the uniformity in reprocubility of synthesis and coating of the GNPs. Besides, the same amount of Ab was used for coating the GNPs even if they are three months old. It means that synthesized GNPs are stable for long term and can be used for conjugation directly without the needed for new optimization steps for coating. As it is seen in the graph (Figure 26, B), the optimum amount of Ab can be decided as the concentration which produces a minimal decline in absorbance at λ_{max} after the addition of salt. According to the results, if the dilution rate of Ab is high and long incubation time is combined with it (Thobhani et al., 2010), nanoparticle aggregation was occurred even if the pH is optimum. Because high concentration of ionic salt causes the reduction of electrostatic repulsion charges occurred at between the GNPs, and resulted with a bluish grey color flocculation (Chun, 2009).

The UV-Vis measurement of GNPs and conjugates prepared by Salmonella and *E.coli* Ab was demonstrated in Figure 27 and Figure 28, respectively. Although different tools (Lenggoro et al., 2002, Sperling et al., 2007, Kuyper et al., 2006, Boal et al., 2000, Tatumi and Fujihara, 2005, Van Delden et al., 2005) are used for characterization of GNPs solutions, the size, concentration and agglomeration level of them can be estimated by UV-Vis spectroscopy as a useful technique. Because it has short analysis time, the sample is not altered during the analysis and spectra obtained is remained unchanged by changing of pH value (Ao et al., 2006). The λ_{max} of synthesized GNPs and different complexes was measured before and after centrifugal step since the high speed spinning might cause packing of nanoparticles in the solution and resulted by more shifted absorbance (Chenggang et al., 2008). As expected, slightly increased absorbance value and shifted wavelength was observed at conjugated GNPs after centrifugation. Because the absorption of GNPs is changed about 10% by the proteins immobilized on GNPs surface (Dykman et al., 2008). The UV-Vis spectrum of synthesized GNPs showed a SPR peak at 526 nm and 527 nm before and after centrifugation, respectively (Figure 27, A-B). When BSA is added into the GNPs, the max peak was observed at 526 nm before centrifugation, while it was red shifted by ~ 2 nm, after centrifugation (Figure 27, C-D). This result is meaningful since the size of GNPs could slightly be increased when the presence of BSA on the surface of GNPs

(Ye, 2010). This adsorption is also possible with either an electrostatic attraction with lysine residues (Casals et al., 2010, Brewer et al., 2005) or displacement reaction of thiol ligand through the unpaired cysteine (Tsai et al., 2011), and spontaneous interaction (Dominguez-Medina et al., 2012). However, the maximum absorbance was red shifted by ~6 nm that is 532 nm, in the presence of Ab along with BSA/GNPs solution after spinning (Figure 27, F), while the wavelength of conjugate, e.g. 526 nm was the same with others before centrifugation. This result suggests that both the Ab and BSA interacted with GNPs and this interaction caused both changing of the refractive index at the GNPs surface and shifting of the SPR energy. To highlight, the presence of proteins in wash buffer showed the high absorbance value between the wavelength of 200 nm and 280 nm (Figure 27, B, C, D, E, F) while it was not observed for the naked GNPs (Figure 27, A) as expected. Moreover, the intensity of the protein absorption at 280 nm increased after centrifugation (Figure 27, B, D, F). This result is comparable with Zhao's and Zhang's reports which showed the decrease of the intensity of absorbance at 280 nm caused by removal of the excess unbound Abs or BSA after centrifugal step (Zhao et al., 2010, Zhang et al., 2012). To say that, the absorbance intensity at 280 nm was also found a little higher in the presence of Salmonella Ab along with BSA than in the absence of Ab. This might be shown as evidence that Abs were adsorbed on GNPs and they were not removed with spinning process (Figure 27, B, D, F). The color intensity of the coupled GNPs with Salmonella Abs was also red as expected while it was observed like purplish color caused by aggregation for the naked GNPs after spinning (Figure 27, B, F). Therefore, the Ab adsorption did not induce citrate stabilized GNPs aggregation (Dominguez-Medina et al., 2012) in this work.

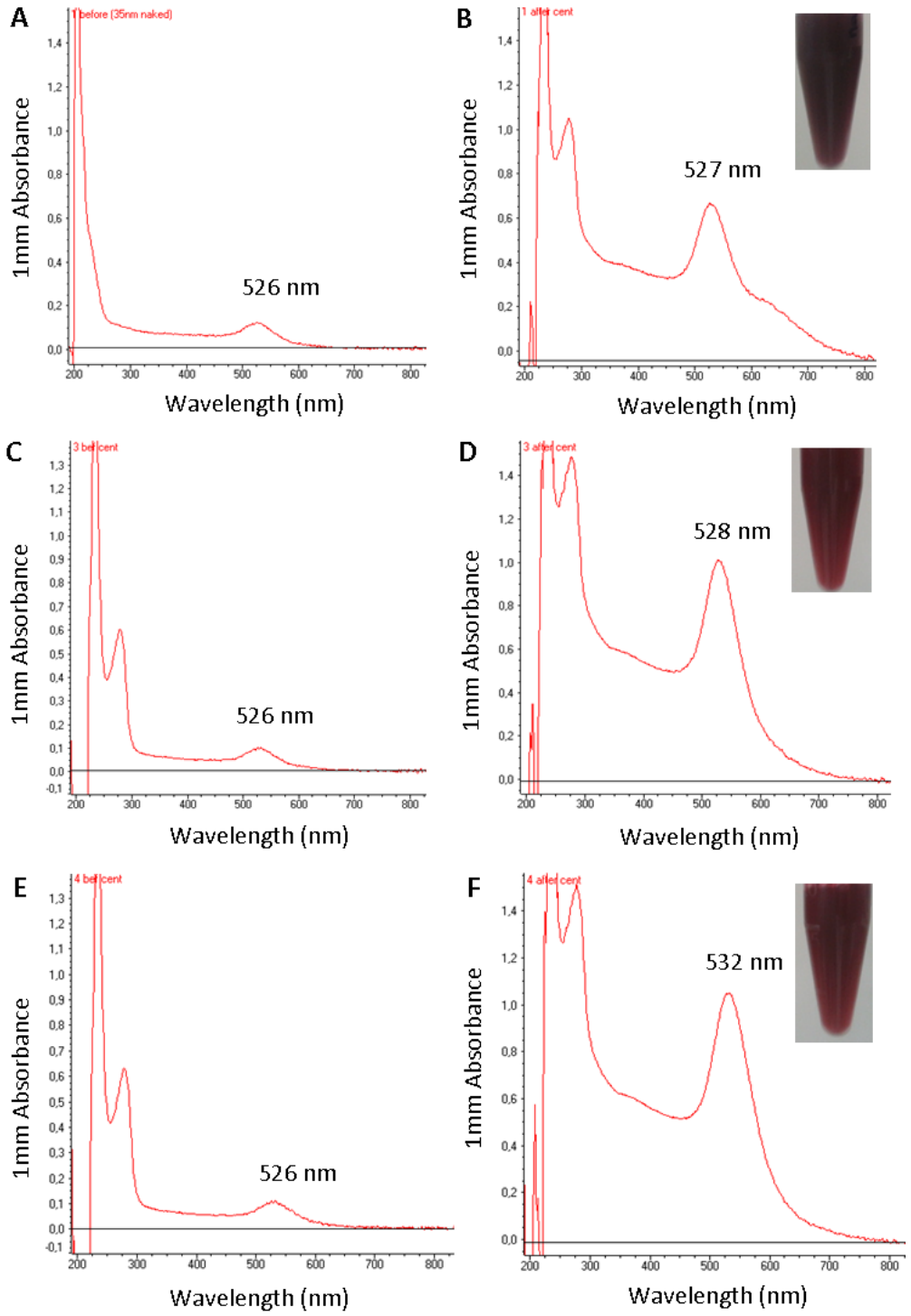


Figure 27. UV-Vis spectrum of naked and conjugated GNPs with/without Salmonella Ab **A)** Naked GNPs before centrifugation **B)** Naked GNPs after centrifugation **C)** BSA/GNPs before centrifugation **D)** BSA/GNPs after centrifugation **E)** Ab/BSA/GNPs before centrifugation **F)** Ab/BSA/GNPs after centrifugation

Figure 28 also depicts the change of UV-Vis spectrum of 36 nm GNPs with and without *E.coli* Ab before and after centrifugal step. All the measurements were reasonable and peak shifting was observed after Ab or protein coating on GNPs as expected because of the explanations mentioned above for the Salmonella Ab conjugate. Briefly, *E.coli* Ab caused the increment of absorbance about 6 nm, 533 nm (Figure 28, F), and prevented GNPs from flocculation. However, naked GNPs were agglomerated and their color was changed from red to purplish in the absence of Abs (Figure 28, B). It is clearly seen that the conjugation methods, method 1 and 2, showed almost the same affect with respect to the absorbance of GNPs conjugates. This means that both methods can be used for coupling of different Abs with GNPs to make a stable conjugates.

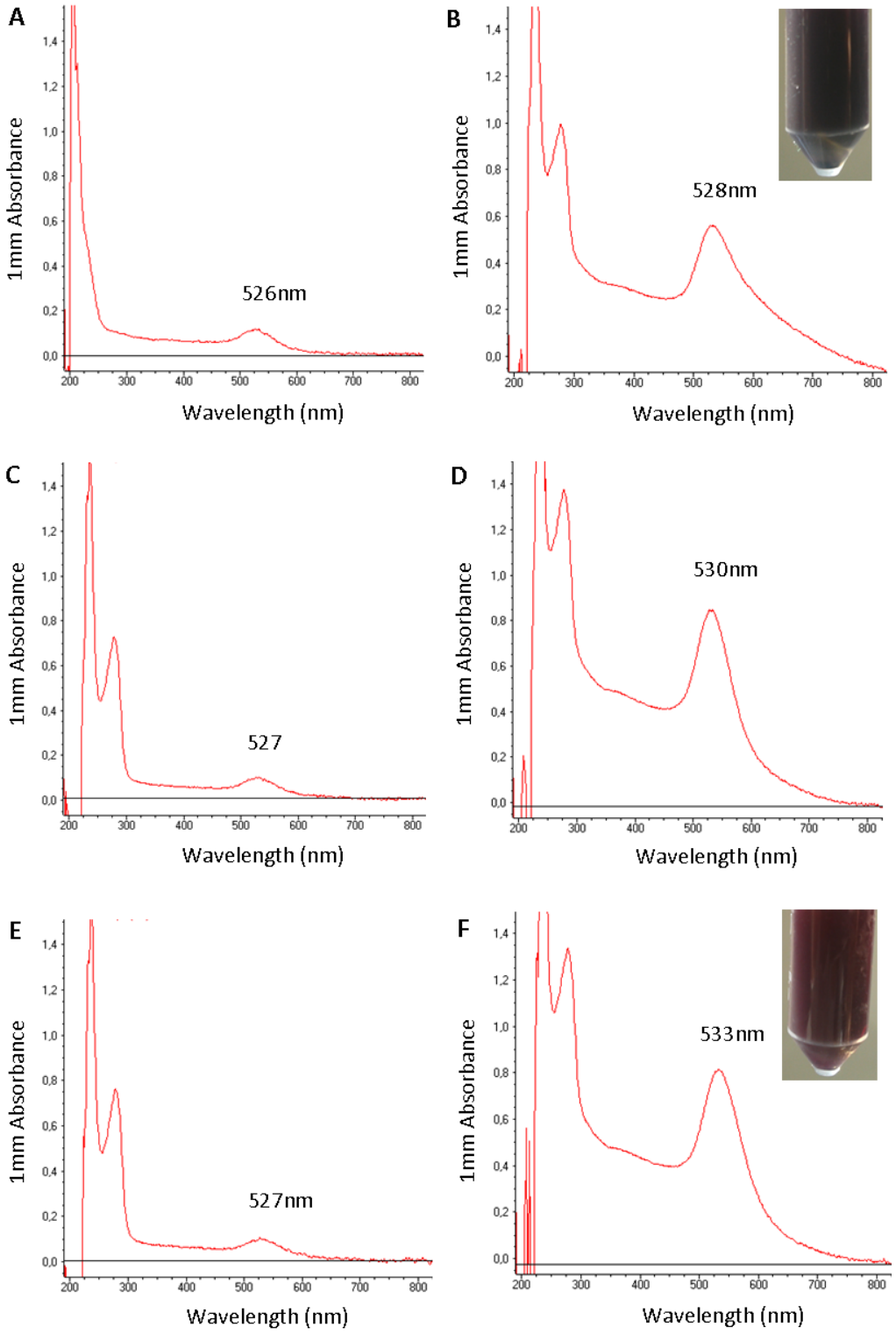


Figure 28. UV-Vis spectrum of naked and conjugated GNPs with/without *E.coli* Ab **A)** Naked GNPs before centrifugation **B)** Naked GNPs after centrifugation **C)** BSA/PEG/GNPs before centrifugation **D)** BSA/PEG/GNPs after centrifugation **E)** Ab/BSA/PEG/GNPs before centrifugation **F)** Ab/BSA/PEG/GNPs after centrifugation

Zeta measurement was preferred for the size evaulation of GNPs since it provides precise and quick measurement. Thus, the size of both synthesized GNPs and Salmonella Ab/GNPs conjugate (conjugate A) was recorded as 36 nm and 87 nm, respectively (Figure 29, A-B). This size should be the maximum for the conjugate since it is not increased by the higher amount of Ab than optimally selected concentration (Thobhani et al., 2010). Both naked and conjugated GNPs had shown perfect size distribution. It might be deduced that Abs were adsorbed with an equal number or intensity onto each GNPs.

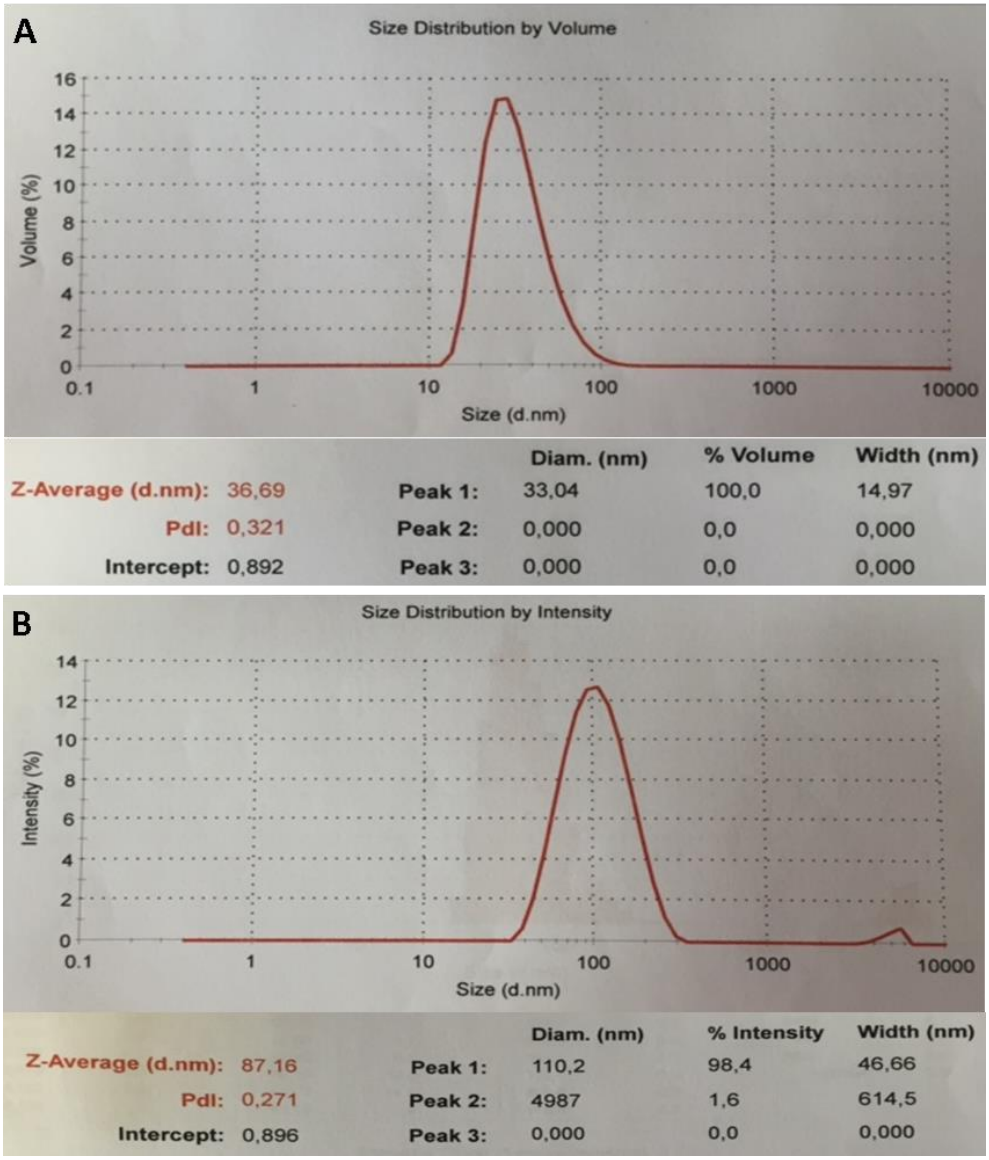


Figure 29. Size distributions of naked GNPs **(A)** and Ab/GNPs conjugate (conjugate A) **(B)**

Zeta potential is the whole charge of particles which is acquired in the specific medium. The stability of the colloidal solutions can be indicated by the magnitude of the Zeta potential. All the particles repel each other if they have a large positive or negative zeta potential and dispersion stability is occured. However, dispersion instability is observed when the low zeta potential is occured on the particles because of the absence of force preventing them from coming together. Since the Zeta potential of conjugate can be changed by different pH values (Ao et al., 2006) all the measurements were performed at pH: 9. It was -53,9 mV (Figure 30, A) for naked

GNPs and -30 mV for the conjugate (Figure 30, B). However, it was reported as -44,1 mV for the 36 nm GNPs in the literature (Nara et al., 2010). It can be said that both the naked GNPs and conjugate are considerably stable. Because the value of Zeta potential which is more negative than -30 mV or more positive than +30 mV shows the stability (Nara et al., 2010). These results are also in agreement with the report that negative Zeta potential is seen at naked GNPs at pH: 3.6-10, and highly decreased value is observed for the conjugate with increased pH from 3.6- to 10 (Ao et al., 2006). As a result, adsorbing of Abs on the GNPs was resulted with the size increment, and the surface of negatively charged GNPs was found as the less negative caused by the positive charges come from the Abs after coating procedure.

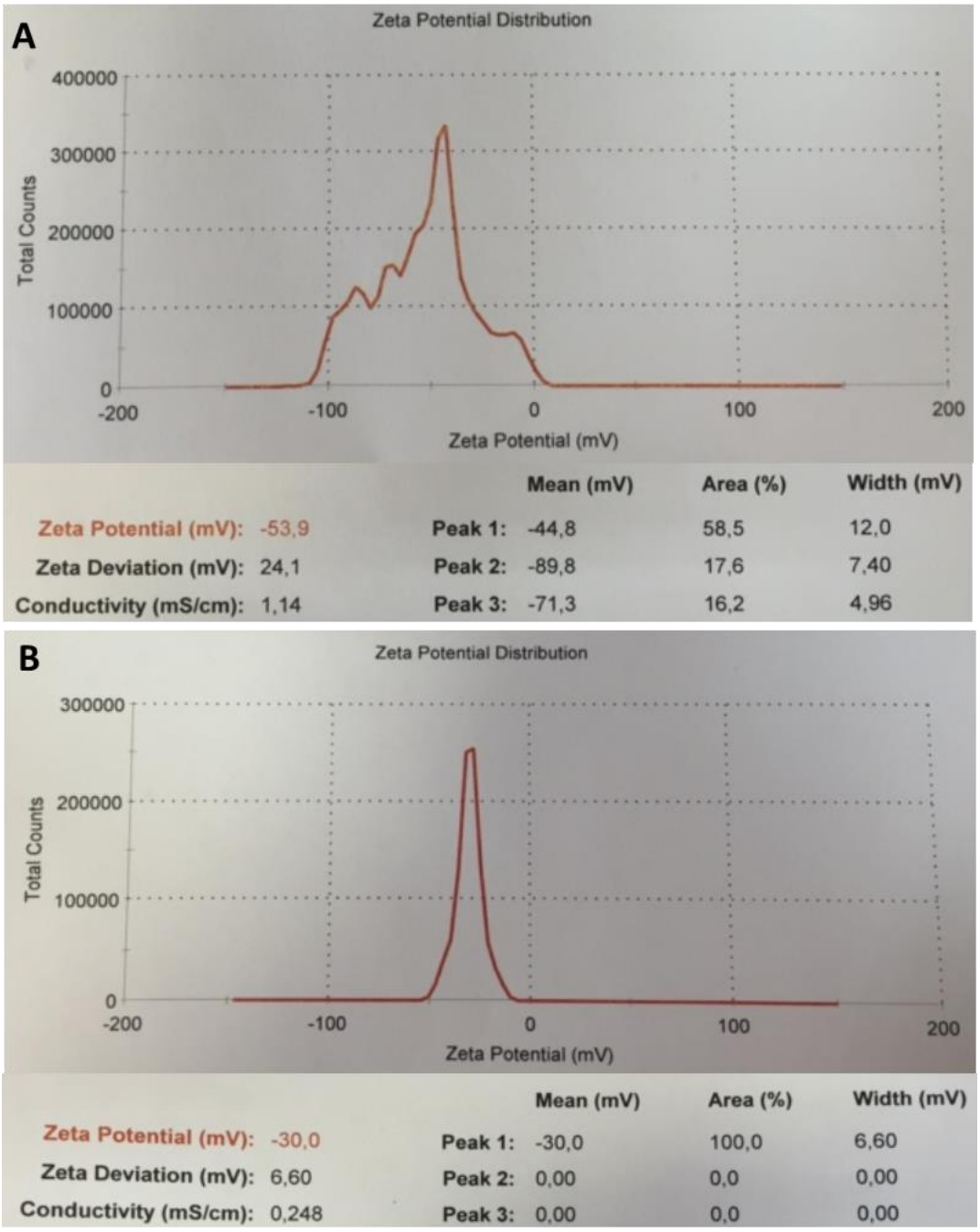


Figure 30. Zeta potential measurements of naked **(A)** and Ab/GNPs conjugate (conjugate A) **(B)**

As a summary, regarding to whole concept of conjugation, pH value, used buffer composition, incubation time and loaded Ab concentration determine the adsorption degree of proteins on GNPs and possible aggregation. In general, the optimal conditions may be differed by the changing of either the Ab or nanoparticles (Thobhani

et al., 2010). For instance, both pH value and optimum Ab concentration were different for the Salmonella and *E.coli* Ab for coupling reaction. However, preparing the conjugates with both scFv and commercial Salmonella Ab has the same optimal conditions even if different sized GNPs were used for developing the LF assay, in this study.

3.3.2. Designing of Lateral Flow Strips

To start with, NC membrane was adjusted to different width such as 0.4-0.5-0.6 and 0.7 cm by cutting the membrane without loading control line Abs to see the appropriate wideness for strip development (Figure 31, A). Then the optimal distance between the capture lines were also determined by immobilizing the reagents on different remoteness (Figure 31, B). The optimum width for strips was decided as 0.4 cm. It is also clearly seen that if the control line was placed on far away from the test line, capturing efficiency was significantly decreased (Figure 31, B1). If both lines were immobilized on close to the absorbent pad, capturing was weak (Figure 31, B3). Therefore, both lines were spotted on the membrane with a 0.5 cm distance and the initial point of test line was adjusted to 0.4 cm far away from the conjugate pad (Figure 31, B5-B6) for further studies.

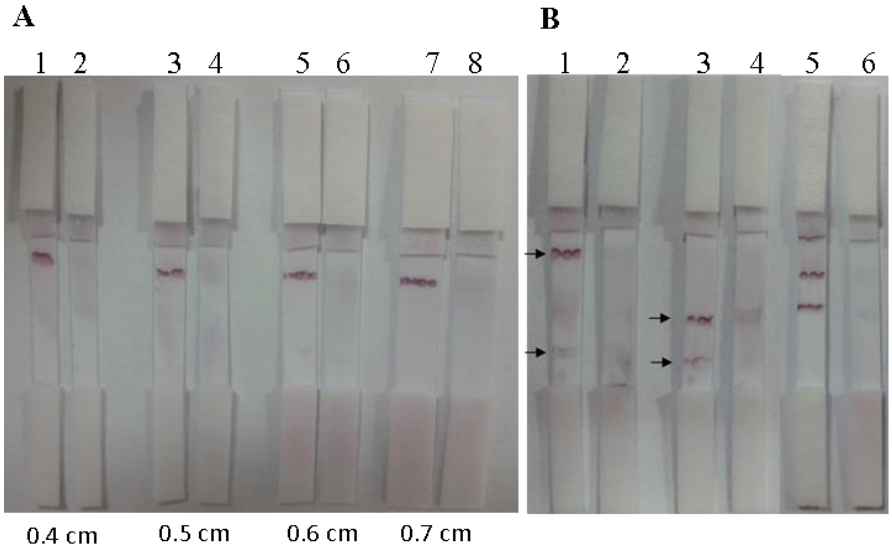


Figure 31. Comparison of strip width **(A)** and distance between the test and control line **(B)** using HF240 membrane. Test line: 0.25 mg/ml *E.coli* Ab **(A, B)**, Control line: 0.25 mg/ml *E.coli* Ab **(B)**. Immobilization was at RT. Distances between two lines are 1 cm **(B1, B2)** and 0.5 cm **(B3, B4, B5, B6).** Strips A2, A4, A6, A8 and B2, B4, B6 were prepared by naked GNPs and 10^6 *E.coli* positive control cells in 100 µl were loaded to the all strips. Arrows indicate the places of capture zones

3.3.2.1. Single Chain Variable Fragments based Strips

scFv stabilized GNPs had been found to good assay specificity. Thus, the detection of *S. typhimurium* by developed LF strips based on scFv/GNPs conjugate was succeeded. Both the conjugate and the affinity of scFv on the GNPs were found to be stable for 3 weeks. To verify capturing of LPS antigens on the strip, two kinds of immobilization were experienced (Figure 32, A). It was observed that LPS antigens were recognized by scFv conjugate without depending on the location of antigens immobilized at either test or control zone. Similarly, capturing efficiency and the color intensity of the lines were found to be independent from the location on the NC membrane (Figure 32, B). Since the orientation of Abs available on the GNPs has significant role in terms of binding to the antigens (Backmann et al., 2005, Peluso et al., 2003) it seems that Ab was oriented suitably and Ab binding site is available for binding target antigens. As expected, any red line was not seen on the strips designed with naked GNPs (Figure 32, A3). When designed strips were dipped into the target samples, 14028s and LT2,

both strains were detected in a 3-5 min (Figure 32, B). This shows the evidence of the presence of scFvs on GNPs and they have still good affinity for binding. In a comparison, aptamer based LF strip developed by 15 nm sized GNPs had ability to recognize the DNA sample of *S. typhimurium* (Rastogi et al., 2012) instead of whole cells. It is clearly seen that target bacteria were recognized by sandwich model occured at between scFv/GNPs conjugate and IgG when it is alone (Figure 32, B1, B3, B5, B6) and included in the mixtured bacteria (Figure 32, B11). However, the interaction between the scFvs on GNPs and scFvs immobilized on capture lines was observed even if negative controls were used as an antigen (Figure 32, B) except for strip B4. Thus, it is not clear that *S. typhimurium* was captured by sandwich model occured at between the scFv and scFv/GNPs conjugate (Figure 32, B1, B3, B5).

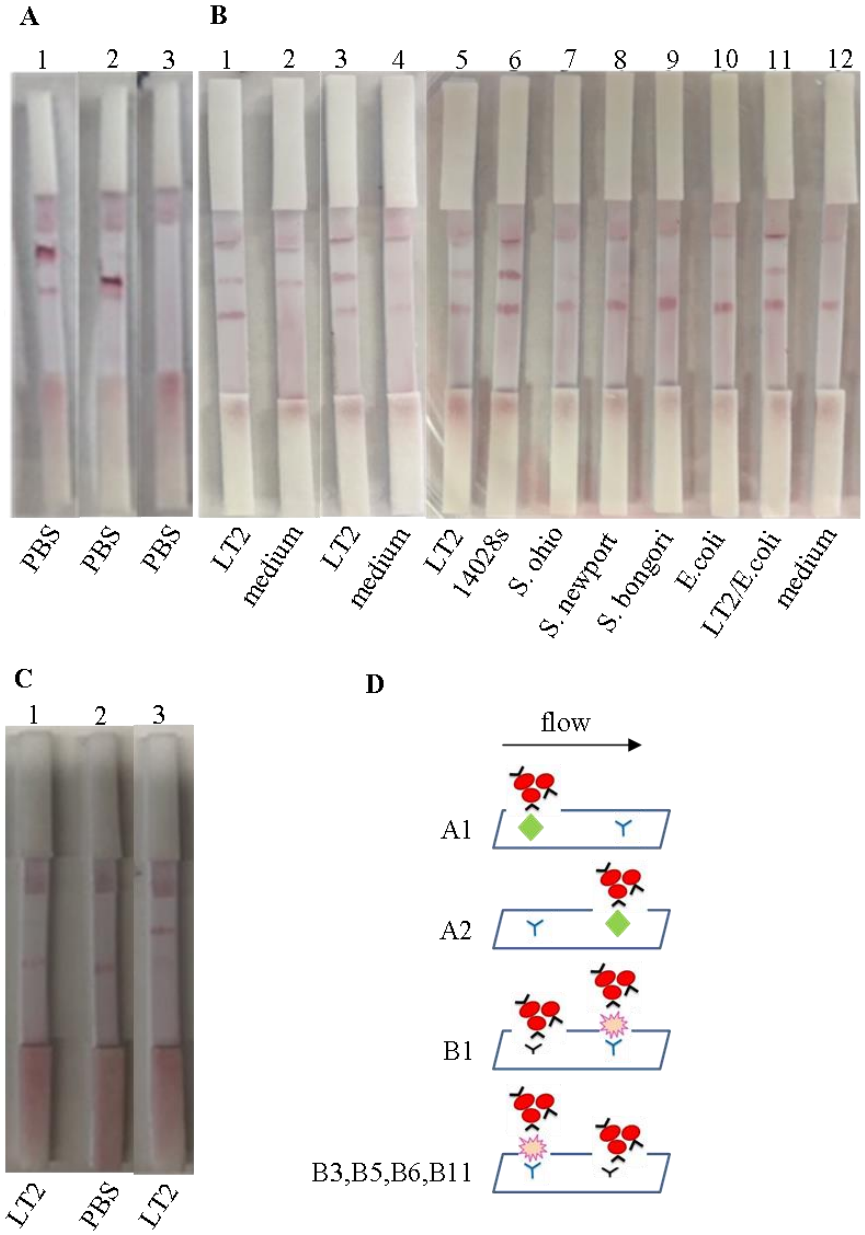


Figure 32. Development of LFIA strips based on scFv using 1X **(A, B)** and 5X GNPs conjugate **(C),** and **D)** representation of positive test results observed at A&B, schematically. HF180 membrane was used to recognize the target. Test and control lines for strip A1: 0.6 µg/strip LPS and antigoat IgG, respectively, strips A2, A3: 0.6 µg/strip antigoat IgG and LPS, respectively, Strips B1, B2: 1.08 µg/strip scFv and 0.6 µg/strip antigoat IgG, respectively, Strips B3-12: 0.6 µg/strip antigoat IgG and 1.08 µg/strip scFv, respectively, Strips C1-2: 0.3 µg/strip antigoat IgG and 0.7 µg/strip scFv, respectively, Strip C3: 1.08 µg/strip scFv and 0.6 µg/strip LPS, respectively. Strips A3, was prepared by naked GNPs. Green square: Serogroup B LPS, pink circle: Live *S. typhimurium.* Blue Ab: Antigoat IgG, Black Ab: scFv

As expected no interaction between the scFv on GNPs and IgG on the capture lines was observed when the presence of non target antigens (Figure 31, A, B, C), respectively. Although 5 times concentrated GNPs, 5X, were also used for developing the test strips, they have not recognized the LT2 strains efficiently (Figure 31, C). This might be caused by decreased binding affinity of scFvs on the concentrated GNPs or weak adsorption of scFv onto GNPs. Application of samples to the strips was not dependent on either loading the samples as droplets (Figure 31, A, C) or dipping the sticks into the test tubes (Figure 31, B). Although the LOD of Salmonella was not experimented with developed strips, it might be enhanced by making further strip assays in regards to the comparison with ELISA results. Because it may be in good aggrement with ELISA as reported (Wang et al., 2013) or may be better than it. It is known that goats, rabbits and mice are well suited for the production of polyclonal Abs in order to develop the LFIA strips (Bailey, 1994, Mikawa et al., 2009). However, engineered scFv and its bacterial expression showed highly suitable alternative for developing immunassay test strips in regards to saving time and making easy to generate Abs in this work.

3.3.2.2. Common Structural Antigenic Antibodies based Strips

In general, colloidal gold Ab particles which are sized as 20 nm and 40 nm are the most utilized sizes in LFIA (Chandler et al., 2000, Fang et al., 2011, Christopher et al., 2005). Because if the GNPs are larger than 40 nm self coagulation and poor stabilization might be seen, and if they are smaller than 10 nm the color of GNPs might not be a bright and clear (Fang et al., 2011, Christopher et al., 2005). Therefore, 36 nm sized GNPs were coupled with commercial Abs for developing LFIA in this work. Both the test and control line Abs were effectively immobilized in PBS onto NC membrane manually without adding any reagent. Because the presence of any reagents such as sodium chloride or methanol may diminish the quality of lines and cause artifacts (Gonzalez et al., 2011). Although various volume of solutions, e.g. 0.75 µl/cm (Zhang et al., 2012), 5-10 µl/cm (Zhang et al., 2011) for the capture lines were used (Liu et al., 2006, Jiang, 2009, Zhang et al., 2012, Xu et al., 2009), it was applied as 0.6 µl/0.4 cm strip which is almost in aggrement with 2 µl/cm (Gonzalez et al., 2011), in

this work. The NC membrane was allowed to incubate at 30˚C for 30 min for efficient immobilization of Abs while it might be performed at 70˚C for 15 min (Nara et al., 2010), 1 hr at 40˚C (Zhao et al., 2008) and overnight (Gonzalez et al., 2011). Using special buffers for blocking the sample and conjugate pads is crucial to ensure the clear band occurance and effective flow for developing immunochromatogrophic assay. Sample pad buffer used in this work had shown positive effect on flow, and it may also be prepared with various components (Choi et al., 2010, Zenget al., 2009) according to the analyte tested. Since the drying time for pads has no obvious effect on the test (Zhang et al., 2012) it was not tested in this work. While loading the sample into the sample pad in different volumes, e.g. 50-250 µl (Zeng et al., 2009, Zhao et al., 2008, Rastogi et al., 2012, Ang et al., 2012, Gonzalez et al., 2011, Xu et al., 2009) is possible, 100 µl of sample was run in this work. Increased sample volume was resulted by decreased color intensity on the lines, which is similar with no growth of signal on the test zone by higher sample volume than 100 µl in Xu's report (Xu et al., 2009). The reason may be that completion of the reaction between Ab and antigen in a very quick time is being suffered from solid-phase sandwich based LF assay. Another reason might be an amount of sample which will pass through the lines freely without interaction of immobilized capture agents when it is too high (Xu et al., 2009). On the contrary, flow was witheld when the sample was loaded in 50 µl volume.

3.3.3. Optimization parameters of developed Lateral Flow Strips

3.3.3.1. Antibody concentrations on the capture zones

Immobilization of capturing Abs on the lines were examined at various conditions (Figure 33). Initially, heat treated Salmonella positive control cells were immobilized on the control line in order to make sure that Salmonella Abs on the GNPs are able to capture the target, while 0.15 µg Salmonella Ab per strip is immobilized on the test line. Nonspecific interactions on the capture zones were observed under some conditions (Figure 33, A2, B2, C2) except for 37˚C for 30 min incubation time. Thus, it was decided as optimal condition for further development of test strips (Figure 33, D3-D7).

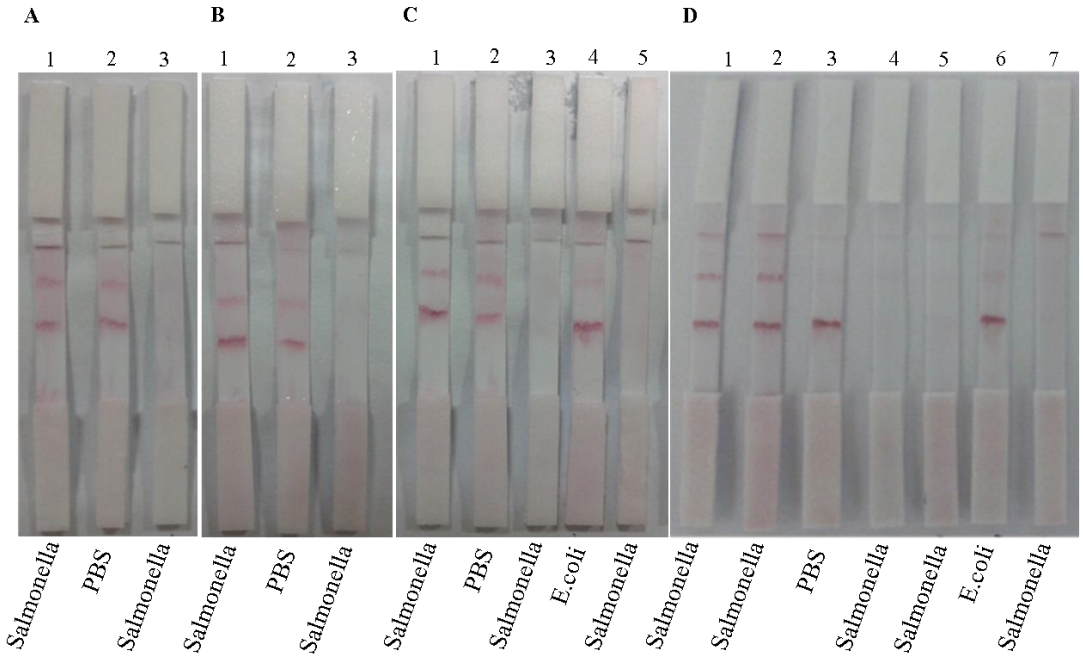


Figure 33. Determination of optimum temperature for the immobilization of capture lines on HF240 membrane **A)** 37˚C for 80 min **B)** 45˚C for 1 hr **C)** RT for 30 min **D)** 37˚C for 30 min. Test line: 0.15 µg Salmonella Ab/strip, Control line: $3x10^6$ Salmonella positive control cells. Strips A3, B3, C3, D4 were designed with naked 1 GNPs, strip D5 was prepared with naked 3 GNPs come from Table 15. Strips C5 and D7 have no capture Abs on both lines. $5x10^6$ Salmonella positive control cells and $3x10^6$ *E.coli* positive control cells in 100 µl PBS was loaded on sample pad and results were observed in 5 min

After deciding the optimal temperature and incubation time for immobilizing the capture reagents, different Salmonella Ab concentrations were experienced on test line. Optimum Ab concentration on the test line was chosen as 0.3 µg/strip for both Salmonella positive control cells and live Salmonella cells (Figure 34 A, Figure 35 A). These amounts had stronger red color on the test zone than others, and they were low when compared to the 1 g/l (Tripathi et al., 2012), 1 mg/ml (Choi et al., 2010, Lou et al., 2012), 0.25-0.5 mg/ml (Zhang et al., 2012) and 8 µg/cm (Yu et al., 2011) capture Ab on test line. Moreover, the comparison of HF240 and HF180 using the same parameters has also showed that there is not any significant difference in terms of the sensitivity. However, flow rate takes extra 3-5 min for HF240 compared to HF180 (Figure 34, Figure 35).

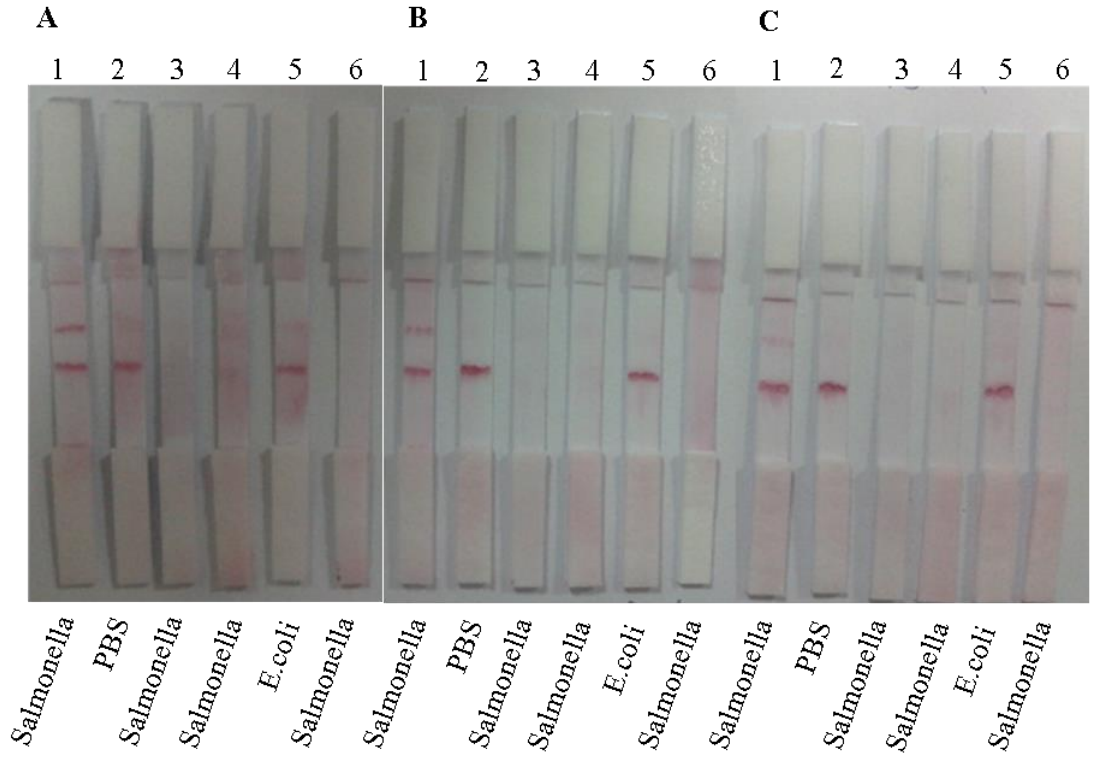


Figure 34. Decrease of Salmonella Ab concentration on the test zone of HF240 membrane. Test line: 0.3 µg/strip Ab **(A)**, 0.15 µg/strip Ab **(B)** and 0.1 µg/strip Ab **(C)**. Control line: $5x10^6$ Salmonella positive control cells. Strips 3-4 **(A, B, C)** were prepared with naked GNPs 1 come from Table 15. Strips 6 **(A, B, C)** have no capture Abs on each line. $5x10^6$ Salmonella positive control cells and $3x10^6$ *E.coli* positive control cells in 100 µl PBS was loaded on sample pad

Additionally, isolates provided by Ankara University, Faculty of Veterinary Medicine and our laboratory strains were captured by different amounts of Ab on the test zone of HF180 membrane (Figure 35). Although Salmonella capturing either alone or in a bacterial mixture sample was succesful, control lines of some strips were not seen (Figure 35, A3-A5, A7, B4, C4-C6). According to the results shown in Figure 35, 0.3 µg Ab/strip on the test zone seems to be enough for capturing live Salmonella cells without any non specific binding. Besides, there was no significant difference between the experienced higher amount of Ab on test lines (Figure 35, A, B, C). However, non specific binding was observed on the test lines of strips shown in B2, C1, C2 for negative controls when 0.5 and 0.6 µg Ab/strip used on test lines. It can be said that excess amount of Ab on the test line and bacterial medium might cause the background

signals. *S. enteritidis* 1 was captured sensitively and selectively when it is alone and in the mixture (Figure 35, A6, A8). It might be related with sample preparation, non-effected flow rate by media and Ab specifity to this strain. *S. infantis* 1 was also recognized in the mixture sensitively and tests seem to valid (Figure 35, A9, C8). Although it was captured on test line when it is alone, tests seem to invalid because of the absence of the control line (Figure 35 A7, B15, C4). It might be caused by very high number cells loaded on sample pad and all conjugates interacted with loaded cells. Thus, it was resulted by not enough conjugates for interacting with control zones. In general, unefficient sample flow was the main reason for unexpected control lines due to clumping caused by intensive media on the conjugate pad.

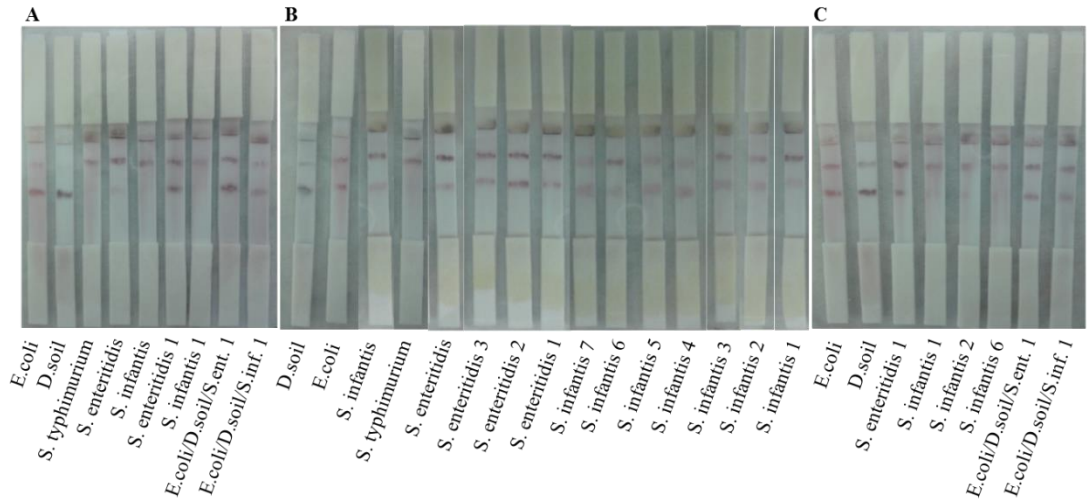


Figure 35. Different Ab concentrations on the test lines for capturing of live Salmonella using HF180 membrane in a 10 min by dipstick assay. Test line: 0.3 µg/strip Ab (**A)**, 0.5 µg/strip Ab **(B)** and 0.6 µg/strip Ab **(C)**. Control line: $5x10^6$ Salmonella positive control cells. D.soil: Dry soil bacteria sample. 500 µl overnight cultures were transferred into the eppendorf tubes separately for dip stick assay. The mixture of different bacteria was prepared by adding 500 µl of each into the mix and after mixing the cultures homogenously, 500 µl of mix was transferred into the tubes for dipping the strips

Briefly, experimented amount of Abs on the test line was reasonable for developing LF assay for Salmonella. Therefore, higher Ab concentration in the test line was not used since it may be resulted with high background signal or decreased

immunoreaction efficiency with a higher Ab (Zeng et al., 2009).

In terms of the developed strips for *E.coli* O157:H7, 0.15 µg/strip Ab was chosen as optimal Ab concentration on the test line since there is no significant difference between 0.21 µg/strip and 0.15 µg/strip Ab (Figure 36, A1-B1, A2-B2). Compared to Zhao's study this amount was remarkably low (Zhao et al., 2010). When the amount of Ab is lowered, the color intensity on the line become weak as expected. Figure 36 also demonstrates the results obtained after 5 min without a PBS wash step (A1-E1) and a 10 min result with PBS washing (A2-E2). Although capture lines become visible clearly and sensitively in a very short time, PBS wash showed further increase of the color intensity (A2-E2). Thus, strips were applied by PBS wash for further experiments. Designed strips had no non-specific binding with non-target samples, and as expected strips prepared by naked GNPs did not have any line or background signals on the capture zones.

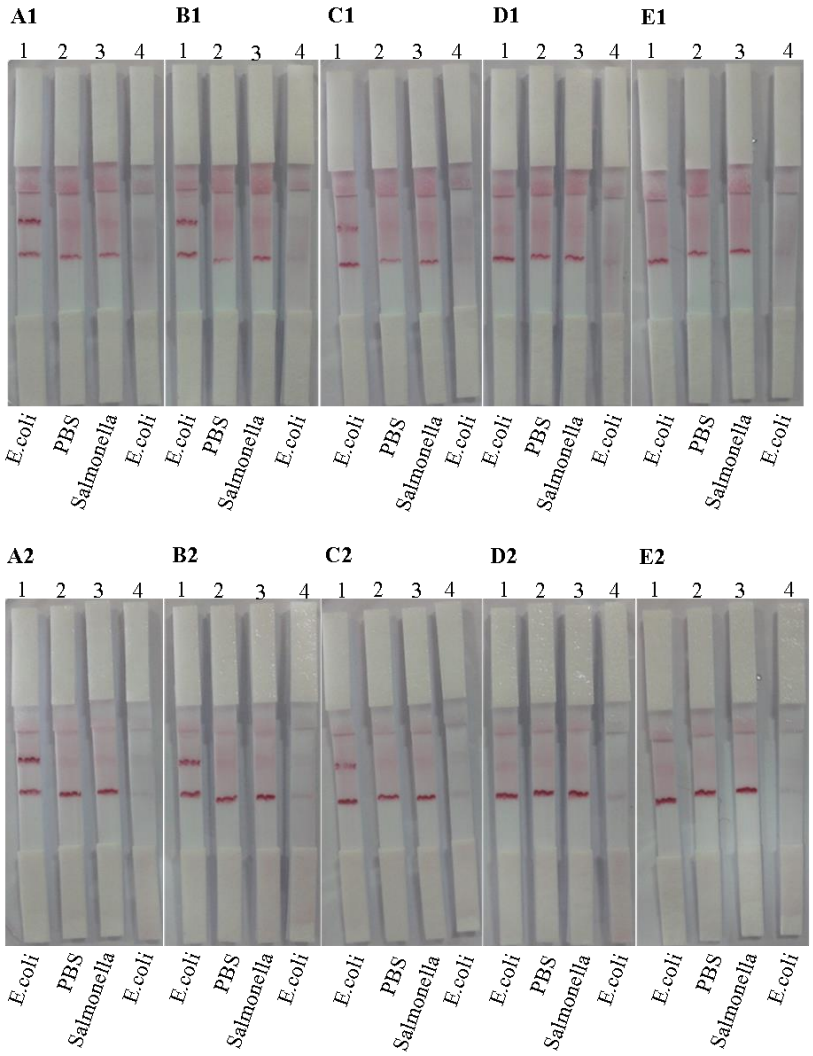


Figure 36. Different Ab concentrations on the test lines for capturing of *E.coli* O157:H7 using HF180 membrane. Test line: 0.21-0.15-0.09-0.044-0.03 µg/strip *E.coli* O157:H7 Ab for **A, B, C, D, E** respectively. Control line: 0.6 µg/strip antiogat IgG. **A1-E1:** Test results after 5 min without PBS wash. **A2-E2:** Test results of the same strips after 10 min with PBS wash. Strips 4 were prepared with naked 1 GNPs comes from Table 16. $3x10^5$ *E.coli* positive control cells and $5x10^6$ Salmonella positive control cells were run in 100 µl PBS

Figure 37 also displays the different concentrations of antigoat IgG on control line in order to find the best amount for developing test strips. The amount of both conjugate and capture Abs was found to proportional to each other in terms of the line intensity, and prepared conjugate had a good intensity on control line with both 0.6 and 0.3 µg/strip IgG for naked eye analysis. However, 0.6 µg/strip IgG concentration seemed

better (Figure 37, A) for *E.coli* strips. Besides, when the amount of Ab is decreased, the color intensity of lines was weakened (C) as expected since the lower number of loaded Ab interacted with lower conjugate particles. Therefore, 0.6 µg/strip antigoat IgG Ab was used for further test development for *E.coli* O157:H7 and also for Salmonella. This amount is also very low compared to the 10 mg/ml IgG with 0,5 µl/dot as application volume (Lou et al., 2012) and 1.0 mg/ml of anti mouse Ab with a volume of 1 µl/1mm line (Zhao et al., 2010). To stress, the concentrations of Abs loaded on the test and control zone were the best amount with respect to the identical color intensity of both lines for naked eye analysis (Figure 36, B1-B2 and Figure 37, A).

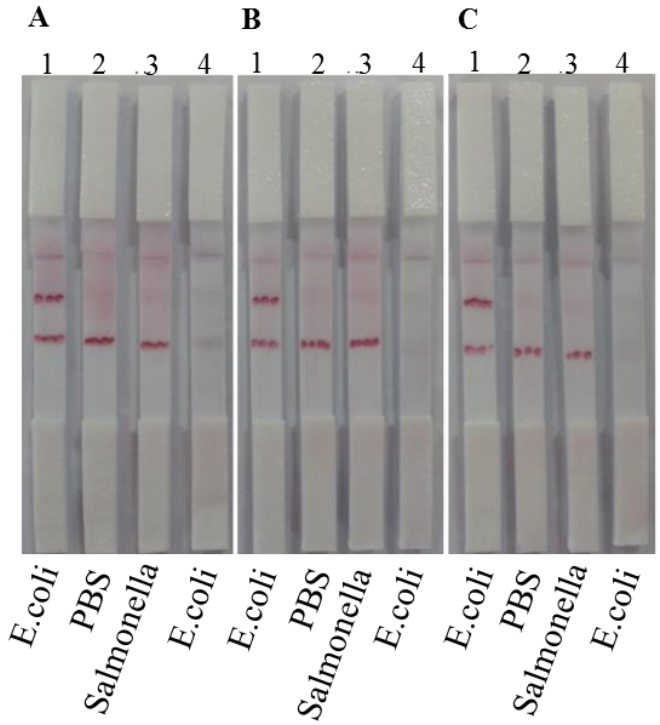


Figure 37. Decrease of Ab concentration on the control zone of HF180 membrane. Test line: 0.15 µg/strip *E.coli* O157:H7 Ab. Control line: 0.6 µg/strip **(A)**, 0.3 µg/strip **(B)** and 0.15 µg/strip antiogat IgG **(C)**. **A-C:** Test results after 10 min with PBS wash. Strips 4 were prepared with naked 1 GNPs come from Table 16. $3x10^5$ *E.coli* positive control cells and $5x10^6$ Salmonella positive control cells were run in 100 µl PBS

3.3.3.2. Conjugate solution on the conjugate pad

The composition of buffer required for the immobilization of GNPs/Ab conjugate is crucial for ensuring the activity of the conjugate. During the assay flow, the binding affinity of Ab present on GNPs surface should be preserved and the flow rate of conjugate from the conjugate pad should not also be affected after drying process. The buffer used in this work was found to be suitable for this purpose and did not affect the activity of conjugate and flow rate of the assay. However, it rarely caused collapse at the end of the conjugate pad when dipsticks were tested in bacterial medium. In Figure 38, various concentrated gold conjugates which are from high concentration to low were prepared and used for soaking the conjugate pads. It was observed that concentrated conjugates retarded the flow on strips and resulted with weak line intensity and nonspecific adsorption of gold conjugate to the NC membrane (strips 1-2). This is reasonable as it is known that the color intensity of the bands occured on both capture zones is proportional to GNPs/Ab conjugate and depends on the amount of conjugate presents on conjugate pad. If greater amounts of conjugate are loaded on the conjugate pad, it can be resulted with longer release time (Ang et al., 2012, Zeng et al., 2009) and insufficient detection. As expected the lower concentration of gold conjugate also showed weak binding to the target on test zones (strips 7, 10). Thus, conjugate A was used for further strip experiments to detect Salmonella.

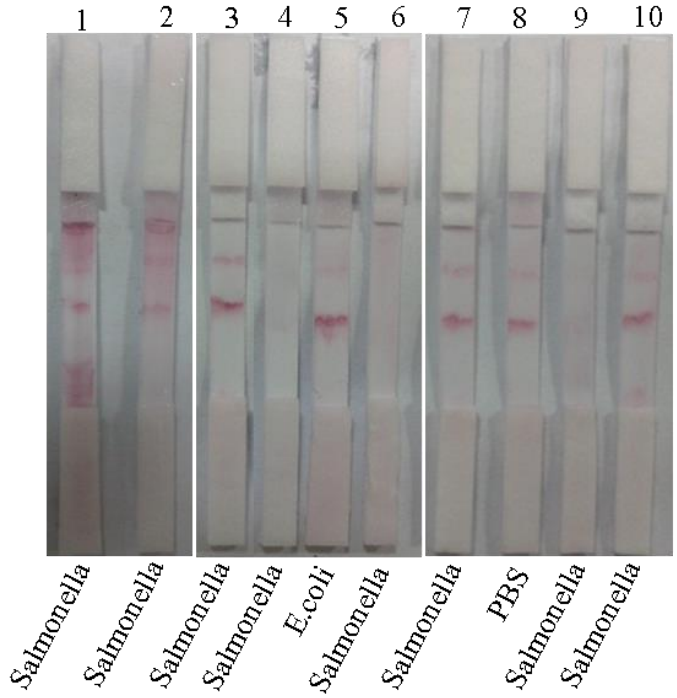


Figure 38. The comparison of conjugate pads prepared by different concentrations of gold conjugate using HF240 membrane for Salmonella detection. Test line: 0.15 µg/strip Salmonella Ab. Control line: $3x10^6$ Salmonella positive cells. Strip 1: prepared by conjugate B (5 times concentrated conjugate), strip 2: prepared by conjugate C (2.5 times concentrated conjugate), strips 3-6: prepared by conjugate A (normally prepared conjugate), strips 7-10: prepared by Conjugate D (diluted conjugate A with a ratio of ¼). Strips 1, 2, 6, 7, 9 and strips 3, 4, 10 were loaded with $5x10^6$ and $5x10^5$ Salmonella positive control cells in 100 µl, respectively. Strips 4 and 9 were prepared by naked 1 GNPs come from Table 15

In Figure 39 and 40, it can also be seen both the comparison of capturing efficiency and the stability of the conjugates prepared at different times using old and freshly synthesized GNPs for Salmonella detection. *S. enteritidis* and *S. enteritidis* 1 was detected on the test line separately with almost at the same sensitivity and selectivity in a 5 min assay. Thus, developed strips do not need any extra signal amplification on the capture zones (Choi, et al., 2010). It is worth noting that repeating of all experiments at different days gave the same results with the same quality of color on the lines. The background signal of conjugates and non-specific interaction with nontarget cells was not observed (A3, A4, B2, B3, C3, C4). Naked GNPs did not show non-specific binding to both capture zones (strips 5).

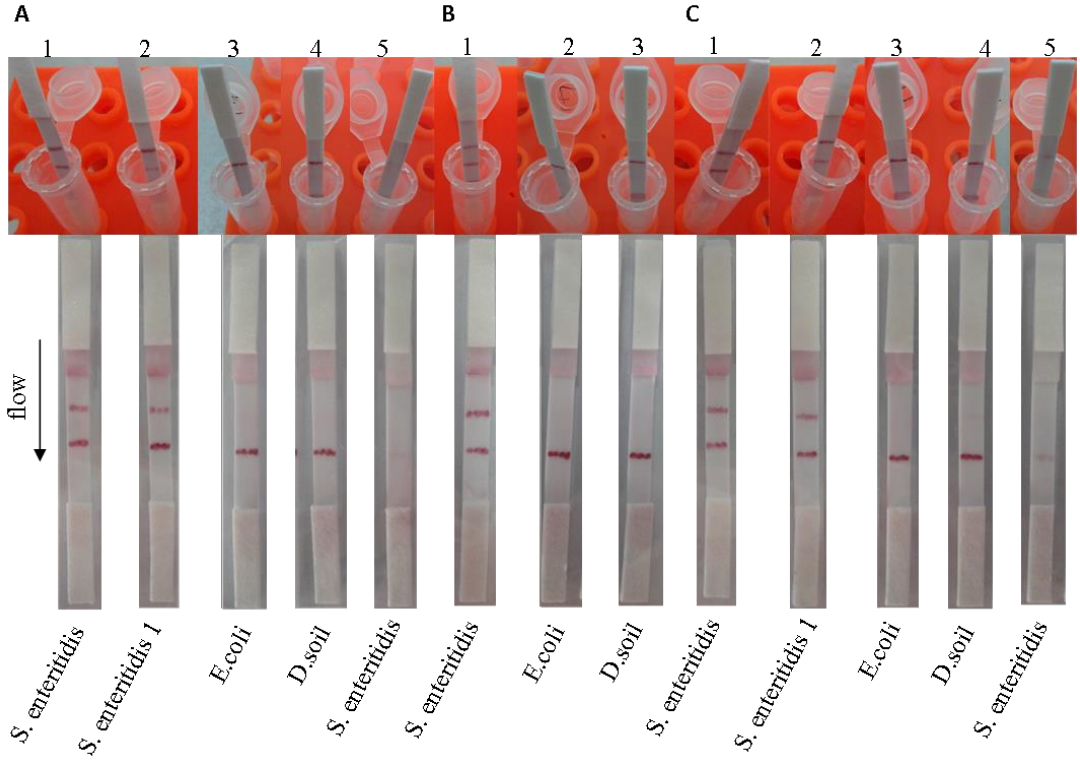


Figure 39. The comparison of gold/Ab conjugates prepared in different times with the same method (method 1) using HF180 membrane. Test line: 0.3 µg/strip Salmonella Ab. Control line: 0.6 µg/strip anti goat IgG. Sample preparation was performed by mixing 100 µl of overnight culture and 100 µl of PBS. The conjugates were prepared by 5, 6 and 2 months old GNPs for **A, B** and **C,** respectively and called as conjugate 1, conjugate 2 and conjugate 3. Strips 5 are prepared by naked 1 GNPs come from Table 15

Additionally, another strip test results obtained from conjugate 4, which was prepared by 8 months old GNPs were demonstrated in Figure 40. In these strips not only bacteria alone but also bacteria in the mixture were tested with live cells. It was observed that this conjugate has the capability to detect the target bacteria present in the mixture specifically except for the *S. infantis* 1 (strip 3). This might be resulted by clumping on the conjugate pad and unefficient flow through the lines. The color intensity on the control lines seemed as the same for the strips ranged from 10 to 22, but it was intensive for the strips ranged from 1 to 9. It might be caused by the abundant conjugate coming through the test zone as a result of not holding the conjugate on the test line. When compared to strips 10-22, since the lower target cell number was loaded on strips 1-9,

the number of conjugate particles interacted with those cells were also low and it was resulted by weak accumulation of conjugate particles on test zone and more accumulation of free particles on control zone.

According to these results, all the Salmonella strains used were detected specifically by the developed strips and the conjugate seemed to have higher affinity to *S. enteritidis* strains and *S. infantis*. Strips loaded either with the mixture that does not have target (strips 1, 2, 19, 20) or bacteria alone (strips 10-14) did not cause clumping on the conjugate pad.

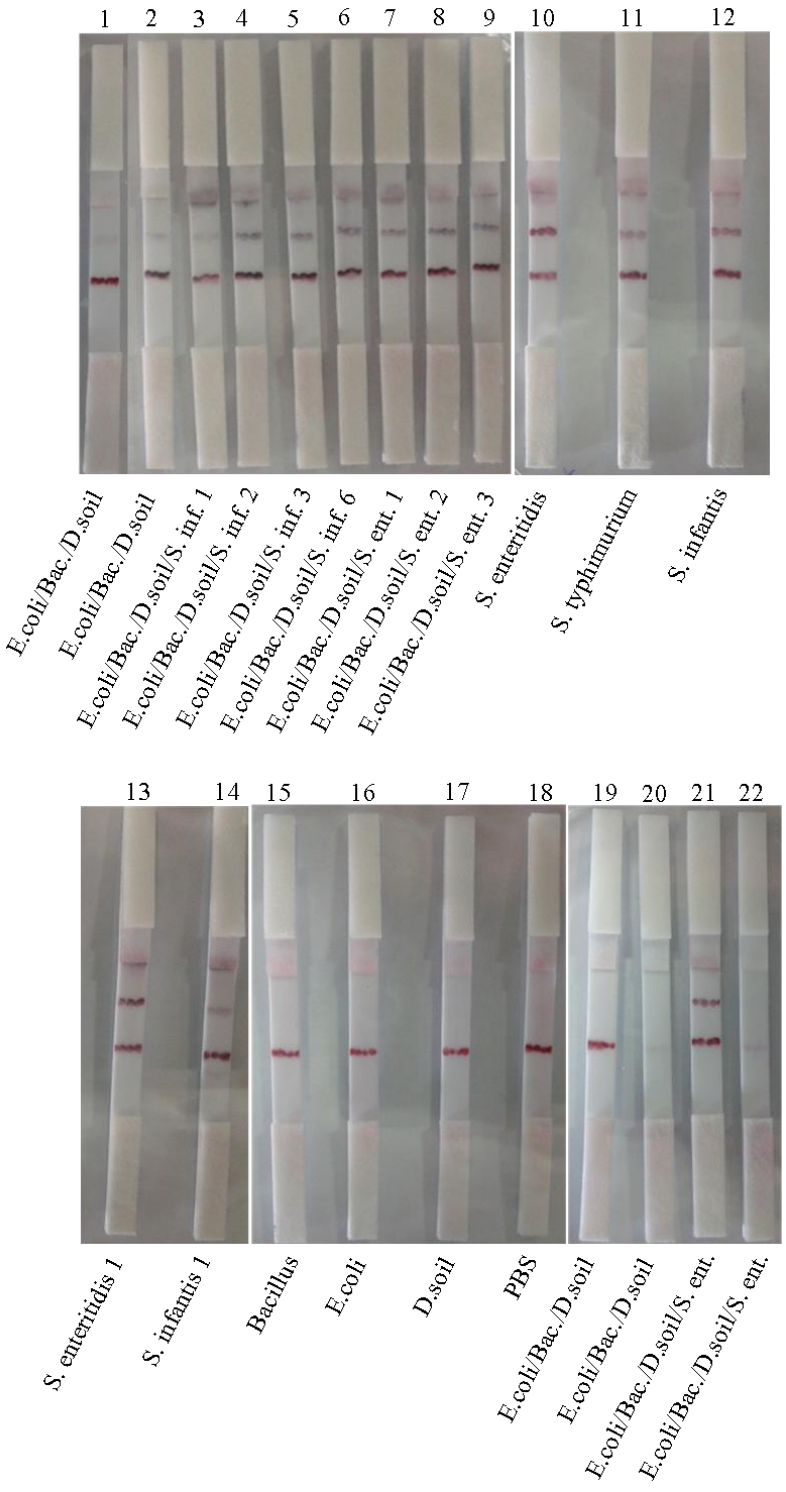


Figure 40. Capturing Salmonella bacteria on HF180 membrane using conjugate 4. Test line: 0.3 µg/strip Salmonella Ab. Control line: 0.6 µg/strip anti goat IgG. Sample was loaded by adding 33 µl and 25 µl of each overnight growth bacteria into 100 µl PBS as total volume separetly for the mixture containing three and four different bacteria samples, respectively. Bacteria alone was loaded as 100 µl from overnight culture. Strips 20 and 22 were prepared by naked 1 GNPs come from Table 15

Figure 41 also demonstrates the efficiency of conjugate concentration on conjugate pad for recognizing *E.coli* O157:H7. Briefly, conjugate A1 seemed to an ideal conjugate for developing the *E.coli* test strips. Because diluted conjugates caused decreased color intensity on both capture lines even if they still have specifity on the strips (A, B, C). Expectedly, nonspecific binding was not observed with negative controls, e.g. PBS, Salmonella and naked GNPs.

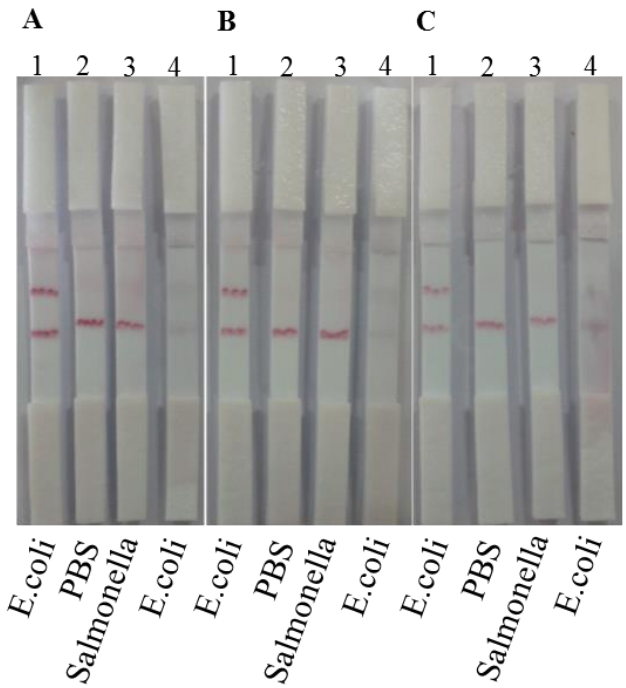


Figure 41. The comparison of conjugate pads prepared by different concentrations of gold conjugate using HF180 membrane for *E.coli* O157:H7 detection. **A)** Conjugate A1, **B)** Conjugate B1, **C)** Conjugate C1. Test line: 0.15 µg/strip *E.coli* O157:H7 Ab. Control line: 0.6 µg/strip antigoat IgG. **A-C:** Test results after 10 min with PBS wash. Strips 4 were prepared with naked 2 GNPs come from Table 16. $3x10^5$ *E.coli* positive control cells and $5x10^6$ Salmonella positive control cells were loaded

3.3.3.3. Comparison of the membrane types

In order to make a comparison between different types of membranes for test development, both positive control cells (Figure 42, A-B) and cultured live Salmonella cells (Figure 42, C-D) were used. It was clearly observed that using both types of cells

did not show any significant difference on the capturing zones. While negative samples were not captured on the test line, targets were detected specifically and sensitively. As it is seen in the Figure 42, there is no significant difference between the membrane types in terms of capturing the target. However, with respect to the releasing of conjugates from the conjugate pads HF120 and HF075 (C, D) were not efficient as HF240 and HF180 (A, B). Thus, HF180 was used for further developing tests since HF240 has very slow flow rate and better for nucleic acid based LF strip (Mao et al., 2009). The non specific adsorption on all the membranes was not also observed. Thus, the developed test strips are positive in terms of the capturing of targets such as Salmonella positive control cells, *S. enteritidis* and *S. enteritidis* 1.

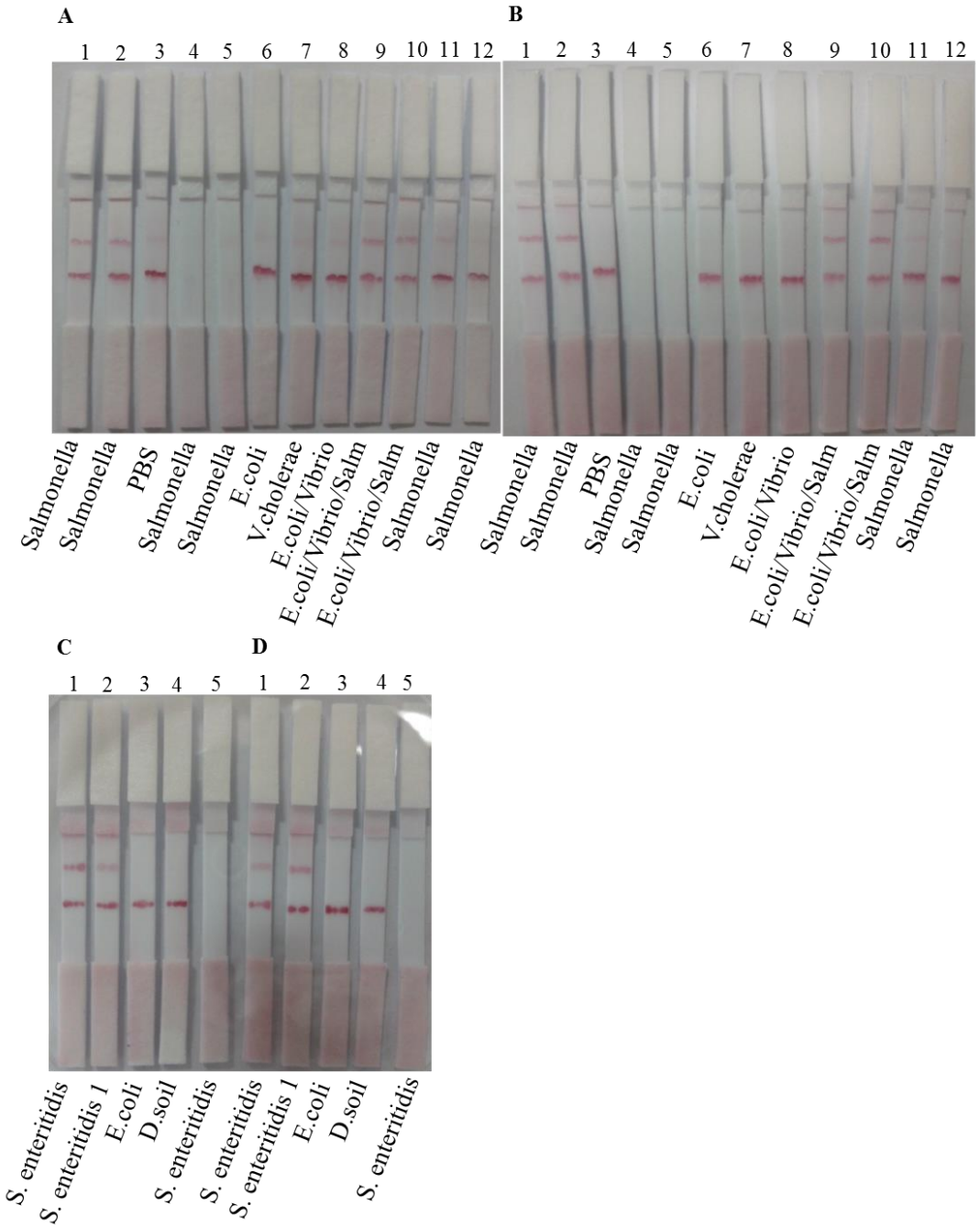


Figure 42. The comparison of membrane types to develop the test strips for Salmonella. **A)** HF240 **B)** HF180 **C)** HF120 **D)** HF075. Test line: 0.3 µg/strip Salmonella Ab. Control line: $3x10^6$ Salmonella positive control cells for **A, B** and 0.6 µg/strip antigoat IgG for **C, D**. Strips A4, B4, C5, D5 were prepared with naked 1 GNPs and strips A5, B5 were prepared with naked 3 GNPs come from Table 15. $5x10^6$, $3x10^6$ and $3x10^6$ positive control cells were loaded in 100 µl PBS for Salmonella, *E.coli* and *V.cholerae*, respectively as seperate and in a mixture format. Salm: Salmonella positive control cells. D.soil: Dry soil bacteria sample

Strips with various flow rates were also experienced twice to decide the best membrane type for *E.coli* O157:H7 recognition. Figure 43 displays the detection of target cells when they are alone (A) and in a bacterial mixture (B, C, D). Although all types of membranes have both lines, which means tests are positive for *E.coli* detection, test line on HF075 membrane (D) was not clear. It was observed that flow time of the membranes plays a significant role in development of the test results. For instance, 7 min and 5 min was enough to see the results clearly by HF240 and HF180 (Figure 43, A-B), respectively while 2 min was enough for both HF120 and HF075 (Figure 43, C-D). This issue is seen on the absorption pads of both HF120 and HF075 since they are red colored with accumulated conjugate caused by rapid flow in a short time. The size of GNPs used for conjugates has also a significant role for flow rate. Thus, both HF240 and HF180 seemed to an ideal membrane types for *E.coli* test strips by using 36 nm sized GNPs. However, HF180 was used for further strip development since it has short analysis time and efficient release of conjugate.

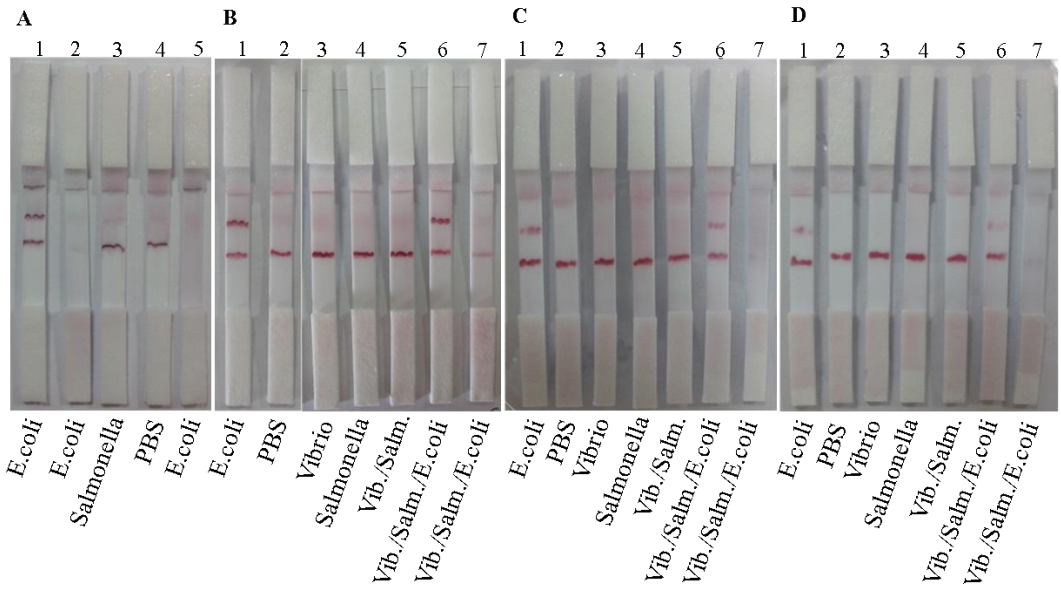


Figure 43. The comparison of membrane types to develop the test strips for *E.coli* O157:H7. **A)** HF240 **B)** HF180 **C)** HF120 and **D)** HF075 membrane. Test results after 5 min **(A, B)** and 2 min **(C, D)** without PBS wash. Test line: 0.15 µg/strip *E.coli* Ab. Control line: 0.6 µg/strip antigoat IgG. Strips A2, B7, C7 and D7 were prepared with naked 1 GNPs come from Table 16. Strip A5 has no capture Abs on the zones. $3x10^5$ *E.coli* O157:H7, $5x10^6$ Salmonella and $3x10^6$ *V. cholerae* positive control cells were loaded in 100 µl PBS. Mixture containing two types of bacteria was prepared by $3x10^6$ *V. cholerae* and $5x10^6$ Salmonella positive control cells in total 100 µl PBS. Mixture containing three types of bacteria was prepared by $3x10^6$ *V. cholerae*, $5x10^6$ Salmonella and $3x10^6$ *E.coli* O157:H7 positive control cells in total 100 µl PBS. Vib: *V. cholerae*, Salm: Salmonella positive control cells

After deciding the membrane type to develop test strips for both Salmonella and *E.coli* O157:H7, their specificity in mixtured bacteria species was further experimented using HF180 membrane (Figure 44, 45). Although 0.3 µg Ab/strip on the test line was decided as optimal Ab concentration for Salmonella, 0.6 µg Ab/strip was also tested to make a comparison in terms of the recognition of Salmonella in the mixture by dipstick assay (Figure 44).

Figure 44-B demonstrates the *S. typhimurium*, *S. enteritidis* and *S. infantis* recognition when they are alone and in the mixture. There was no non-specific interaction with non target analytes and naked GNPs did not react with any reagents on the strips (B4-B5). However, control lines were not seen on strips B1, B2, B10, B12, B15, B16, B19 and B20 while test lines were seen clearly. This was probably caused by inefficient

sample flow from the conjugate pad caused by the high cell density in the applied sample. It is observed that a cell density higher than 10^{12} cells might affect the test results. When compared to the high amount of Ab on test line, 0,6 µg Ab had a better efficiency if the samples are loaded in PBS (Figure 44, B) than in the bacterial medium (Figure 44, A). However, using high amount of capture Ab is unnecessary since it may retard all the conjugate on test line and prevent the clear formation of the control line. As a summary, developed test strips were able to recognize the *S. typhimurium*, *S. enteritidis*, heat treated positive control cells and *S. infantis* (Figure 44, B).

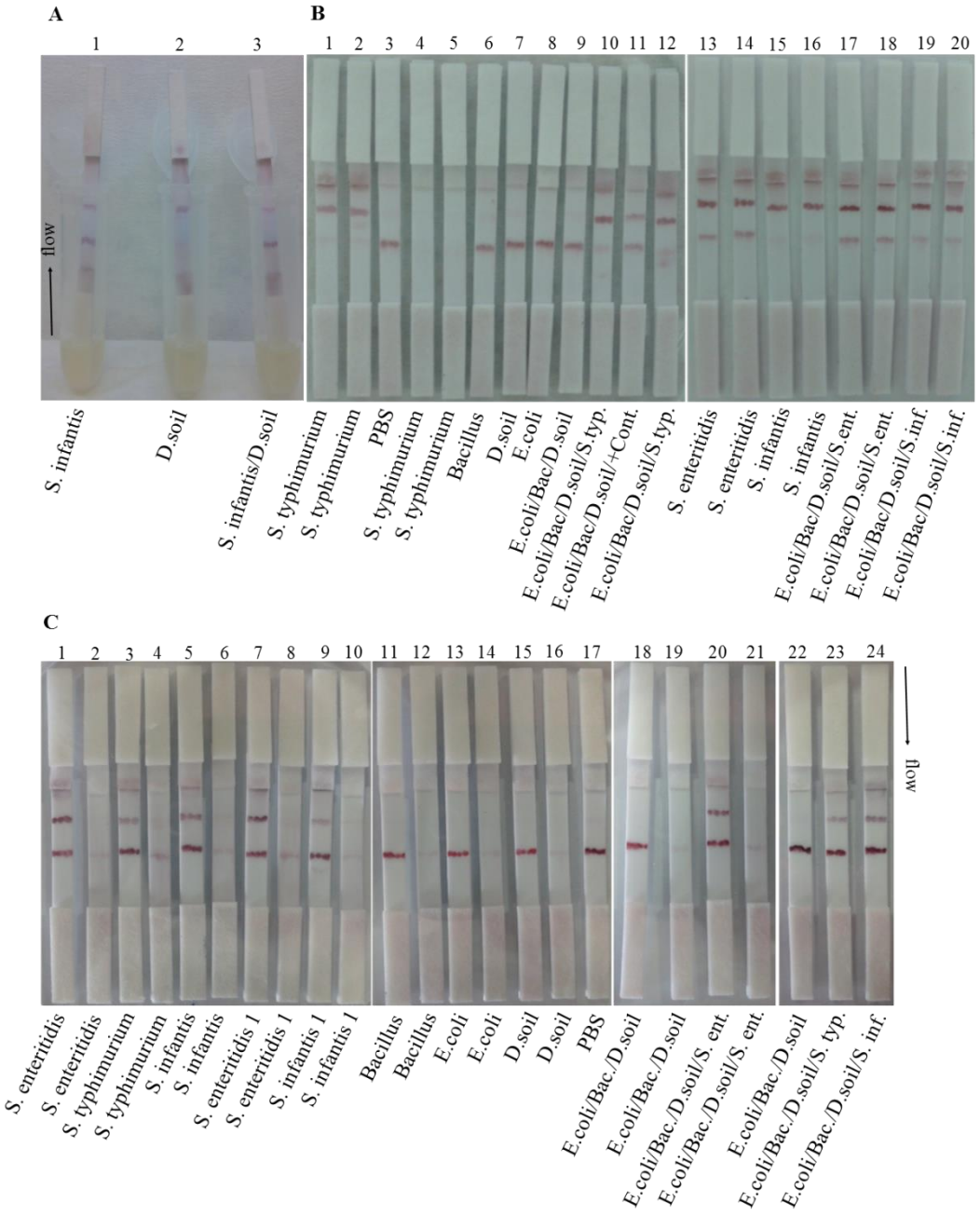


Figure 44. Detection of different live Salmonella strains alone and in the mixture with HF180 membrane after dipstick assay. Test line: 0.6 µg/strip **(A, B)** and 0.3 µg/strip **(C)** Salmonella Ab. Control line: 0.6 µg/strip antigoat IgG. Overnight cultures were applied to the strips. Strips were dipped into 500 µl of total bacteria without adding PBS **(A)** and 200 µl of total bacteria was added in PBS **(B, C)**. Bacteria were used in 100 µl volume when they are alone. Strips B4-C2-C4-C6-C8-C10-C12-C14-C16-C19-C21 and strip B5 were prepared by naked 1 and naked 3 GNPs come from Table 15, respectively. D.soil: Dry soil bacteria, Bac: *B. cereus*, S. ent: *S. enteritidi*s, S. typ: *S. typhimurium*, + cont: Salmonella positive control cells

Figure 44, C also depicts the recognition of Salmonella strians by using 0.3 µg/strip Ab on test line. *S. enteritidis, S. enteritidis* 1, *S. typhimurium and S. infantis* were detected either alone or in the mixture specifically. However, the recognition of *S. enteritidis* and *S. enteritidis* 1 (C1, C7, C20) was better than *S. typhimurium* and *S. infantis* under both contidions (C3, C5, C23, C24), while *S.infantis* 1 was not captured on test line (C9). To highlight, dipping the strips directly into high volume of bacteria in medium (A) affected the flow time and releasing of conjugate from the conjugate pad. However, adding of PBS into the samples (B, C) prevented the agglomeration, and the test results were obtained clearly (C) even if this issue was not observed in some strips (B).

E.coli O157:H7 was also detected in the bacterial mixture by developed strips even in the presence of high concentrations of other bacteria species (Figure 45). When compared to the B1 and B6 strips, the efficiency of capturing on both zones was almost the same with each other, which means that presence of *E.coli* in other bacterial mixture did not affect the test specifity and sensitivity.

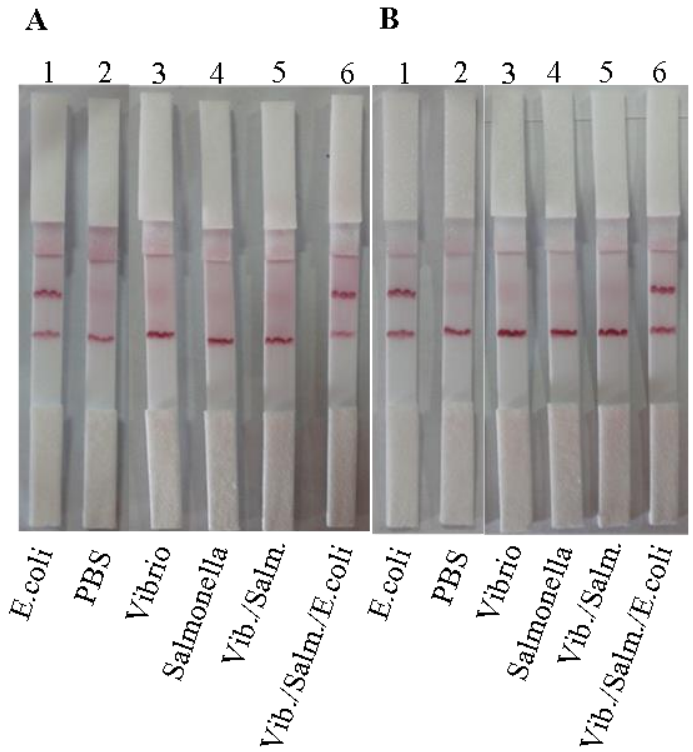


Figure 45. Detection of *E.coli* O157:H7 in the bacterial mixture using HF180 membrane. Test line: 0.15 µg/strip *E.coli* O157:H7 Ab. Control line: 0.6 µg/strip antiogat IgG. Test results after 2 min without PBS wash **(A)** and 10 min with PBS wash **(B)**. Strip 7 was prepared with naked 1 GNPs come from Table 16. $3x10^6$ *E.coli* O157:H7, $3x10^6$ *V. cholerae* and $5x10^6$ Salmonella positive control cells in 100 µl were loaded. Vib: *V. cholerae, Salm:* Salmonella positive control cells

In summary, developed strips had shown applicability to detect the target pathogens, Salmonella and *E.coli* O157:H7, in the bacterial mixture. This result is important with respect to the spesific detection of pathogens in their environment as they are living with various kinds of microorganisms in real life.

3.3.3.4. Limit of detection

HF240 and HF180 membranes were used for determining the detection limit of Salmonella. Although all negative controls among these strips have the same result with Figure 46 D, only one batch was shown in the image. Different membrane types and using increased Ab concentration on the test line did not effect the sensitivity and specifity of detection limit, significantly. LOD of captured Salmonella cells was

determined as $5x10^5$ cells in 100 µl (Figure 46), which are known as enough cell number for gastroenteritis in humans caused by *S. typhimurium* (BVL, 1996). This result is comparable with the detection limit of *S.typhi*, 1.14×10^5 cfu/ml, *S.choleraesuis*, $5x10^5$ cfu/ml, and 10^6 cfu/ml, *S. enteritidis*, by GNPs and gold based immunochromatographic strip using commercial Abs (Preechakasedkit et al., 2012,, Xia et al., 2016, Moongkarndi et al., 2011). Lower cell number of *S. typhimurium* such as 7 cfu/ml (Yuan et al., 2014), $1x10^2$ cfu/ml, $1x10^3$ cfu/ml (Zhang et al., 2006a) and 10^4 cfu/ml (Moongkarndi et al., 2011) was also reported as the LOD by silver enhancement method, biosensor and LF assay, respectively. However, they need to advanced and time consuming experimental steps including biosensor development and nucleic acid isolation of Salmonella for detection by LF assay (Liu et al., 2013) instead of cell surface recognition.

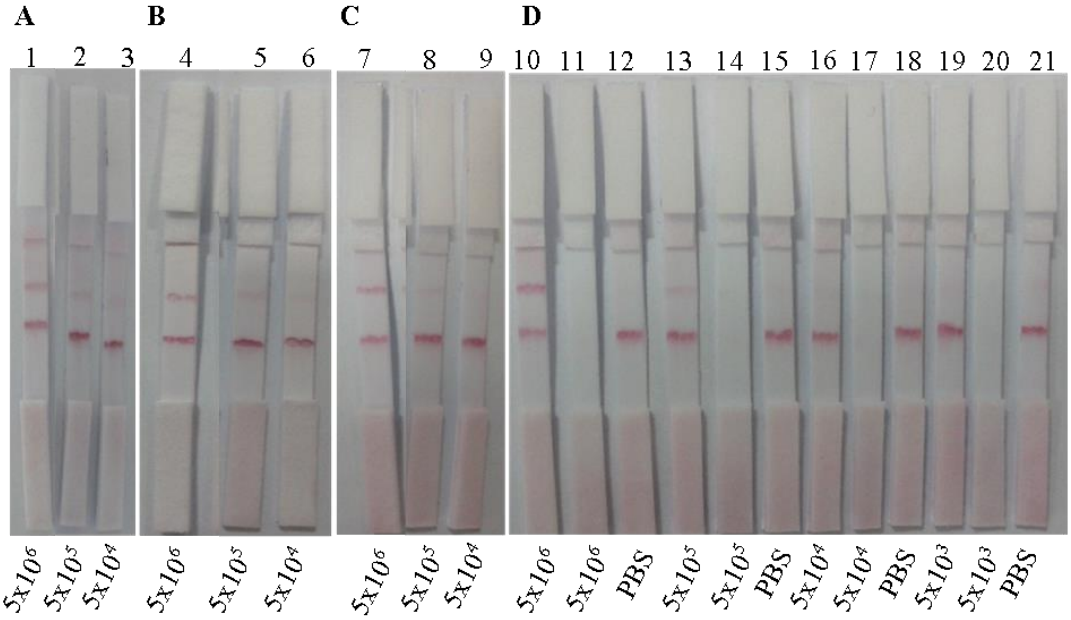


Figure 46. LOD of Salmonella positive control cells by developed LF strips with HF240 **(A, B)** and HF180 membrane **(C, D)**. Test line: 0.3 µg/strip **(A, B, C)** and 0.6 µg/strip Salmonella Ab **(D).** Control line: $3x10^6$ Salmonella positive control cells. Strips shown in **A** were prepared with conjugate 2 and strips shown in **B, C** and **D** were prepared conjugate 4. Strips 11, 14, 17 and 20 were prepared by naked 1 GNPs come from Table 15. From $5x10^6$ to $5x10^3$ Salmonella positive control cells were loaded in 100 µl PBS

Figure 47 displays the LOD of *E.coli* O157:H7. 10^5 cfu/ml was recorded as detection limit (B2). This result was in parallel to the 1.8×10^5 cfu/ml (Jung et al., 2005) detection limit for *E.coli* O157:H7 by colloidal immunochromatographic strip. Besides, strips developed in this study had a lower LOD compared to the 10^6 cfu/ml detection limit recognized by 40 nm sized GNPs based immunochromatographic assay (Suria et al., 2015, Song et al., 2016). Lower cell numbers, 10^2 cfu/ml (Lin et al., 2008), 79 cfu/ml (Muhammad-Tahir and Alocilja, 2003), ~5 cfu/ml (Hossain et al., 2012) and 10^4 cfu/ml (Radke and Alocilja, 2005) were also reported as detection limit of *E.coli* O157:H7 by electrochemical biosensors, conductimetric biosensor, multiplex paper strips and microelectrode array, respectively. However, they are not in easy to use and practical for short time detection compared to this study. Moreover, 10^3 *E.coli* O157:H7 cells were reported as detection limit by flowerlike shaped GNPs based LFAs (Zhang et al., 2015), but the assay needs further preparation steps for GNPs synthesis. Lastly, as the amount of conjugate used is critical to determine the desired cell number, it seemed to an ideal for capturing targets in this study.

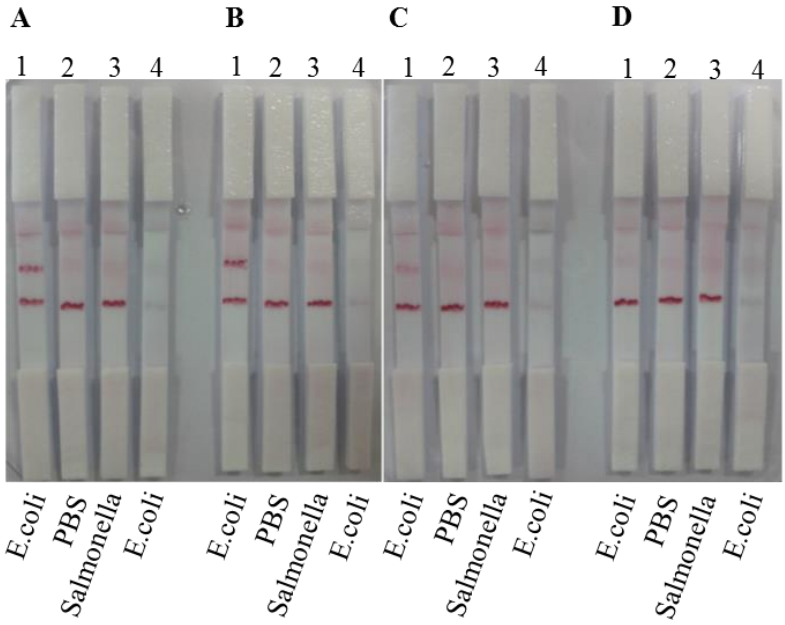


Figure 47. LOD of *E.coli* O157:H7 cells by developed LF strips with HF180 membrane. Strips loaded with 10^6 **(A)**, 10^5 **(B)**, 10^4 **(C)**, 10^3 **(D)** *E.coli* cells in 100 µl PBS, respectively. Test line: 0.15 µg/strip *E.coli* Ab. Control line: 0.6 µg/strip antigoat IgG. Test results after 10 min with PBS wash. Strips 4 were prepared with naked 1 GNPs come from Table 16

3.4. ELISA

3.4.1. Determination of the antigen binding activity of Single Chain Variable Fragments

It was observed that generated scFv has strong affinity to both serogroup B and D antigens, specifically (Figure 48). Comparably, LT2 cells were recognized better than 14028s cells when they are killed or alive. Results showed that the absorbance value of anti Salmonella scFv binding to serogroup B LPS was similar with the binding of *S. typhimurium* DT104 specific monoclonal Ab (Wu et al., 2014) to its LPS. Thus, for the purpose of *S. typhimurium* detection, scFv produced in this study has a potential to be used in LFA strips instead of monoclonal Abs. Coating the microtiter plate with 5 µg/ml LPS was found to have a high affinity by scFv in this study, but BSA conjugated Salmonella O antigen polysaccharide was detected as 10 µg/ml by scFv fusion protein (Wang et al., 2001) of Se155-4 Ab. Although it was reported that se155-4 Ab has very low binding affinity to serogroup D LPS (Bundle et al., 1994b), generated scFv showed strong affinity to this serogroup, which was reported for the first time in this study (Figure 48). This result was considered to be significant since the repeating unit of *S. typhi* (serogroup D) LPS is the same with the serogroup B LPS except for the branch points. *S. ohio* (O7, serogroup C1), *S. newport* (O8, serogroup C2-C3) and *S. bongori* (O66) did not react with scFv as expected because of the different LPS structures (Figure 48). Reproducibility of the binding test to different antigens by ELISA was repeated many times, with consistent outcomes.

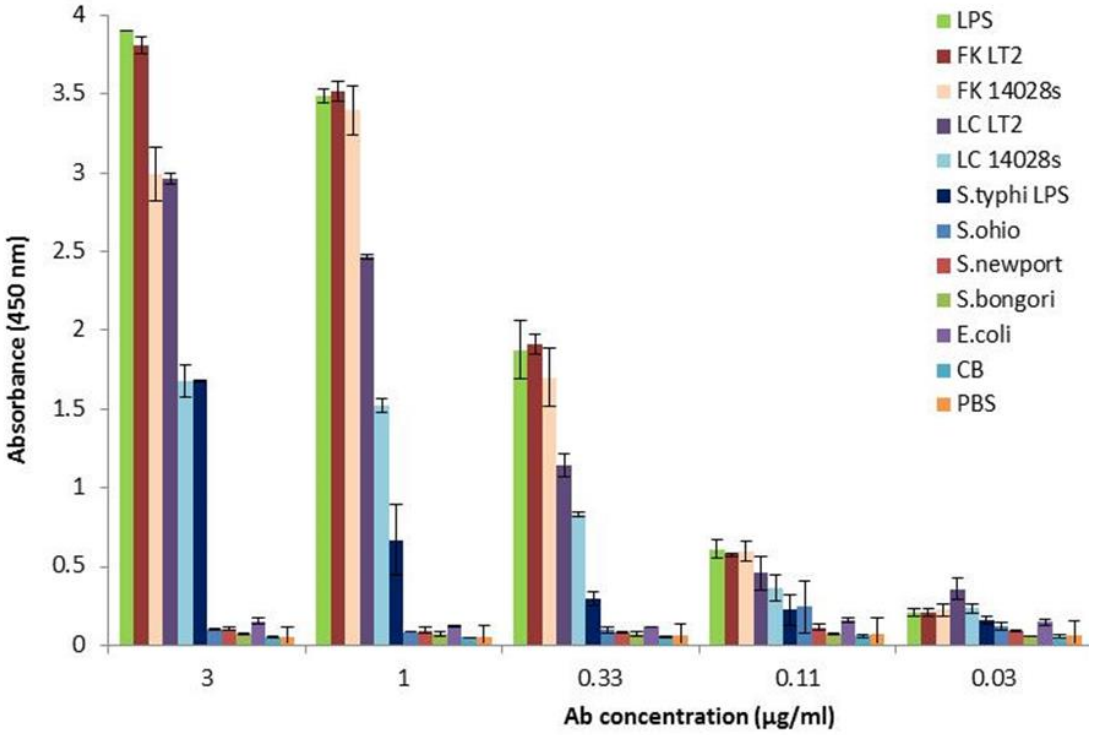


Figure 48. Indirect ELISA results of scFv binding to target and non target bacterial antigens with negative controls. LPS: Lipopolysaccharides from *S. typhimurium*. FK LT2: Formalin killed *S. typhimurium* LT2. FK 14028s: Formalin killed *S. typhimurium* 14028s. LC LT2: Live cells *S. typhimurium* LT2. LC 14028s: Live cells *S. typhimurium* 14028s. CB: Coating buffer

E.coli mediated scFv has a strong affinity to their antigens without cross reaction to *E.coli* or other Salmonella strains in this study. However, in the literature, scFvs against *S. typhimurim* produced by using the naive library showed considerable interaction with live gram positive bacteria and other bacterial species. Additionally, scFvs against *S. enterica* did not demonstrate a high specifity to single bacteria (Cannon and Heinhorst, 2010). To our knowledge, scFv binding to whole Salmonella cells demonstrated in this study is the first record in the literature. Although a few scFvs of se155-4 Ab were produced, the binding affinity of them was shown by using LPS and their different parts (Wang et al., 2001, Anand et al., 1991, Deng et al., 1994) with low affinity.

K_D values of scFv against to different antigens are shown in Table 19. It was obtained by Sigmaplot 10.0 version using the absorbance values come from the ELISA. Results

verified that generated scFv has the highest affinity to target antigens when compared to the values in the literature. This might be caused by the tested antigens and the methods used for the expression or purification of generated scFv.

Table 19. Dissociation constant values (K_D) of scFv Ab against to target&non target antigens

	Kd (nM)	**Std. Error (nM)**
Live cell *S. typhimurium* 14028s	14.2	4.5
Formalin killed *S. typhimurium* 14028s	13.1	6.1
Live cell *S. typhimurium* LT2	22	9
Formalin killed *S. typhimurium* LT2	17.2	4.6
Serogroup B LPS	18.1	4.1
Serogroup D LPS	111.5	18.5
E.coli	N/A	N/A
LPS (-)	N/A	N/A

3.4.2. Detection of *S. typhimurium* in the bacterial mixture

Live cells of *S. typhimurium* LT2 were detected by ELISA in bacterial mixture. To the best of our knowledge, the detection of whole cells of *S. typhimurium* in a mixture by engineered scFv is the first record in the literature. 10^6 live LT2 cells were detected specifically in the mixture with the very close sensitivity of detected bacteria alone (Figure 49, A). When the number of target cells is declined in the mixture, it was observed that 10^5 cells were still detected sensitively. However, 10^4 target cells were not recognized in the mixture while those could be detected when they are alone (Figure 49, B). Using scFv in the ELISA seemed to be an alternative and practical approach when compared to the monoclonal Ab produced by immunization which indicated the detected number of Salmonella cells as 10^7 and 10^5 cells/ml in the presence of *E.coli* (Ng et al., 1996).

The LOD of target determined by indirect ELISA was observed as 10^4 cells in 100 µl by using scFv (Figure 49, B) without enrichment media (Kumar et al., 2008). This is comparable with 10^5 and 10^6 cells/ml *S. typhimurium* detection limit using the hybriodama Ab by direct ELISA (Salam, 2010). Therefore, using scFv seemed to have

sensitivity and advantage for detection of *S. typhimurium.* In Figure 49, it is clearly seen that the target bacteria detection is proportional to the used scFv Ab concentration. Therefore, 0.12 µg/ml scFv can be thought as the minimum amount of Ab to recognize the 10^5 target cells both alone and in the mixture, while 0.37 µg/ml scFv is enough for detecting the 10^4 target cells alone (Figure 49, B).

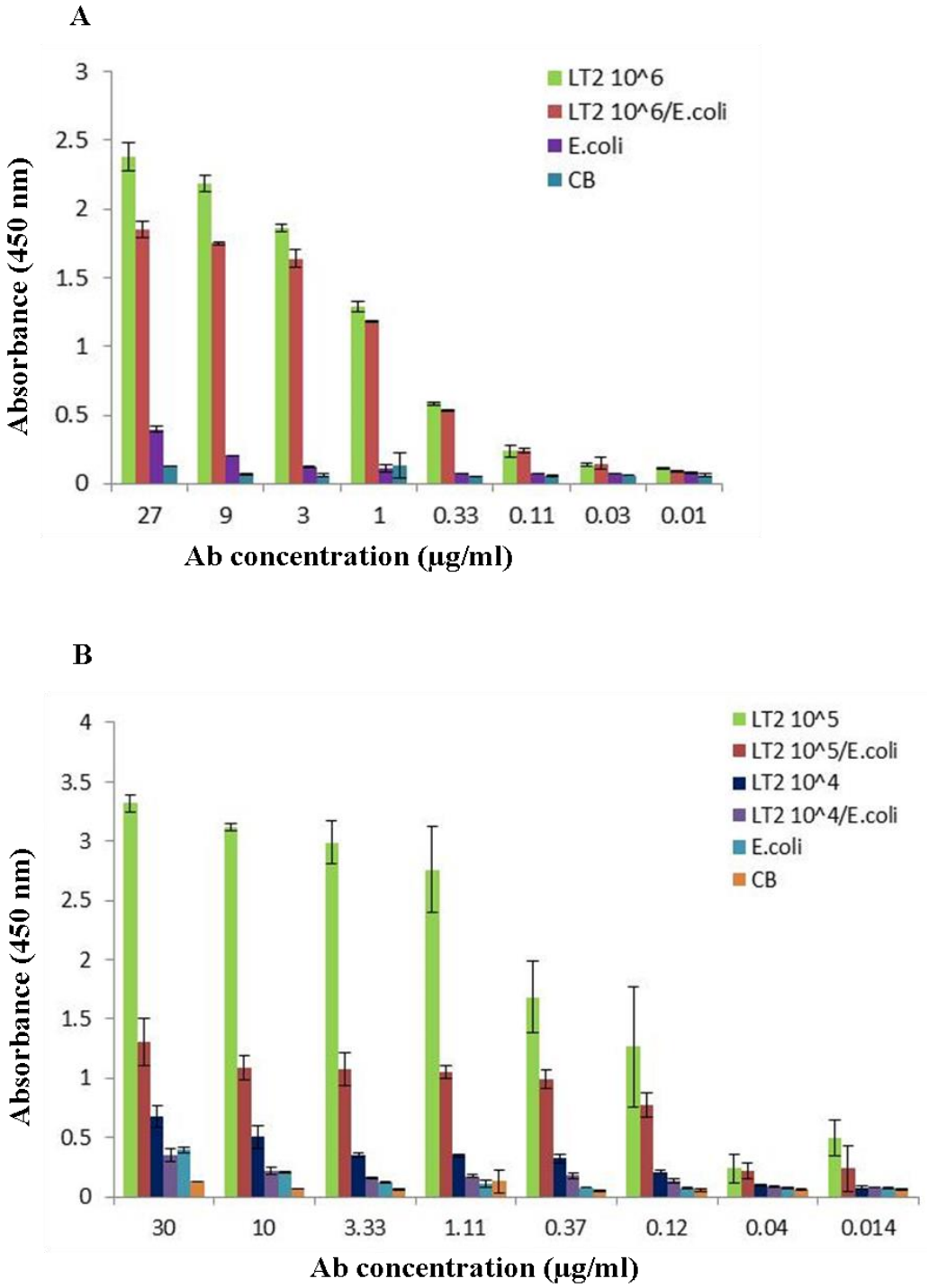


Figure 49. ELISA based live cells of *S. typhimurium* LT2 detection in the mixture by scFv. $2.8x10^6$ LT2 cells **(A)**, $2.8x10^5$ and $2.8x10^4$ LT2 cells **(B)** in 100 µl PBS alone and in the mixture. *E.coli* used: $2.5x10^7$ cells in 100 µl PBS. Total mixture volume is 200 µl. LT2: *S.typhimurium* LT2. CB: Coating buffer

3.4.3. Detection of Salmonella using fusion antibody

The binding affinity of fusion Ab to the serogroup B and D antigenic structures was demonstrated in Figure 50. Even if se155-4 Ab was informed that it had low afinity to Serogroup D (Bundle et al., 1994b), fusion Ab had shown high affinity to them as scFv. It was also surprisingly observed that this binding was highly stronger than the affinity of positive control and callibrator IgGs sepecific to serogroup D antigens supplied in the Kit (Figure 50, A). As expected, fusion Ab has a strong affinity to serogroup B and it is higher than to serogroup D antigens even if lower Ab concentration was used (Figure 50, B, C).

It is clearly seen that, Ab binding affinity to its antigens was proportinal to the Ab concentration used and non specific binding to *E.coli* or uncoated wells was not observed (Figure 50). While 8,8 µg/ml Ab binds to the LPS with a good affinity (Figure 50, B), 4,4 µg/ml Ab has still strong affinity to both LPS and whole cells (Figure 50, C). If compared, the affinity of fusion Ab to LPS of *S. typhimurium* was stronger than whole killed cells (Figure 50, C). This is possible because the interaction of Ab with pure LPS is easier than the antigens available on the cell surface with cellular surface materials. Dissociation constant value of fusion Ab was calculated as 21.9 nM with 3.4 nM standard error after LPS binding assay. As expected, this result is also consistent with the Kd value of produced scFv in this work. Although some studies reported the same affinity of full length Ab and scFv (Pleckaityte et al., 2011), it may also show variety (Wels et al., 1992).

In this work, produced scFv and fusion Ab binding to both LPS and whole cells had about two times higher absorbance value compared to value obtained from monoclonal Ab (Sapparapu, 2003) specific to *S. typhimurium*. Results also verified that engineered fusion protein is humanized Ab. This was demonstrated by using two kinds of commercial Abs which are anti Salmonella CSA Ab and *S. typhimurium* LPS factor 4 Ab. Since those Abs were not humanized and obtained from goat and mouse, respectively they did not interact with the enzyme labelled secondary Ab specific to humanized IgG. However, humanized fusion Ab bound to this secondary Ab (Figure 50, C).

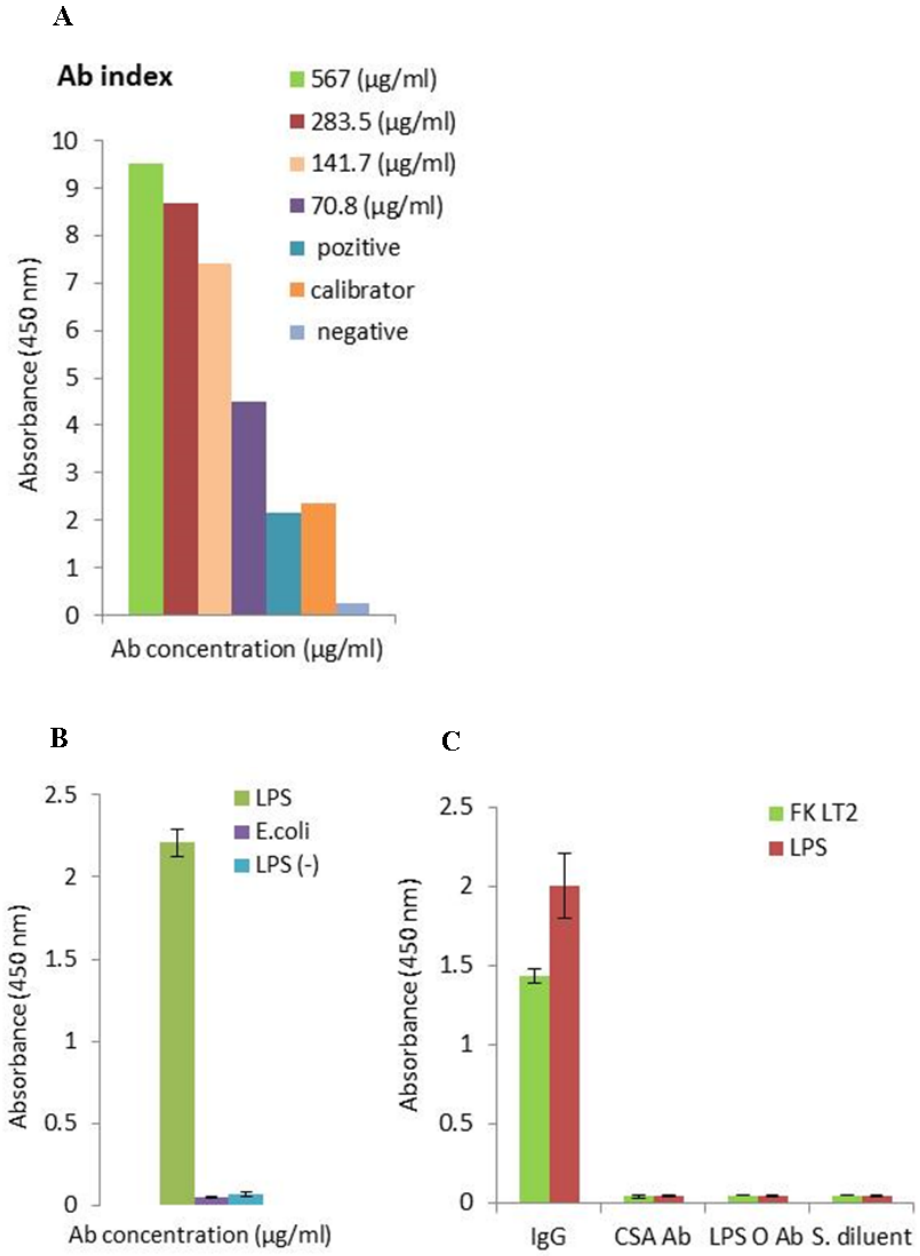


Figure 50. Binding affinity of fusion antibody to serogroup D **(A)** and serogroup B antigens **(B, C)**. 8.8 µg/ml **(B)** and 4.4 µg/ml **(C)** fusion Ab was used. FK LT2 ($4.2x10^8$ cells) and *S. typhimurium* LPS (5 µg/ml) in 100 µl were used for coating the plate, separately for serogroup B binding test. The microtiter plate coated with *S. typhi* LPS (serogroup D) and enzyme labelled secondary Ab were supplied with Salmonella ELISA Kit (Abnova). FK LT2: Formallin killed *S. typhimurium* LT2. LPS: Lipopolysaccharides of Serogroup B. LPS (-): No coating the microtiter plate with LPS. CSA Ab: Anti Salmonella common structural Ab. LPS O Ab: *S. typhimurium* LPS factor 4 Ab. S.diluent: Sample diluent supplied in the Salmonella ELISA Kit. Ab index was calculated according to the manual in the kit for serogroup D binding

3.4.4. Immunomagnetic based ELISA

3.4.4.1. Conjugation of fusion antibody with magnetic beads

Figure 51 demonstrates the coupling of magnetic beads (Promag) with SNAP substrate, BG-GLA-NHS, and interaction of SNAP-Tagged IgG with functional magnetic beads, and also immunomagnetic ELISA in test tubes, schematically. After coupling reaction of Promag beads they were used in a different concentrations for ELISA. Since NEB beads have already SNAP substrates, they were used directly for ELISA.

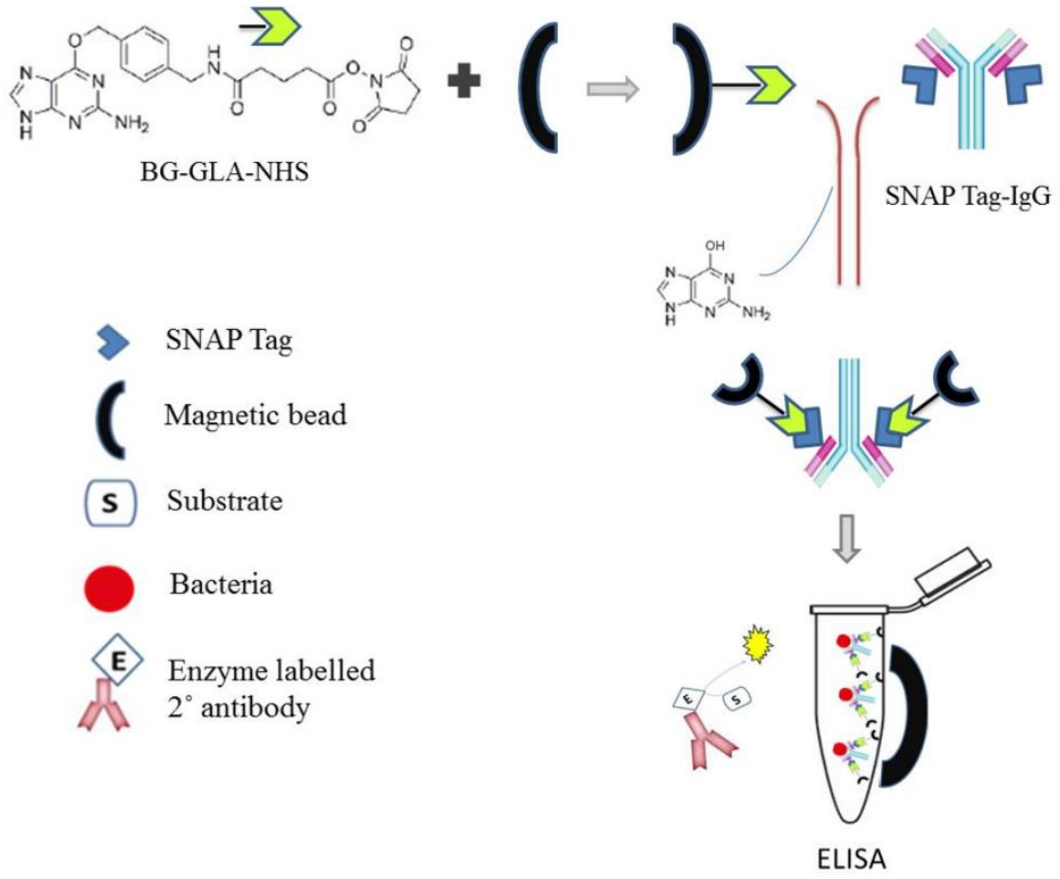


Figure 51. Schematical representation of immunomagnetic capturing of *S. typhimurium* by ELISA

3.4.4.2. Magnetic capturing of *S. typhimurium* by fusion antibody

Magnetic capturing of Salmonella was reported by different methods including GNPs based aptamer/Ab sandwich ELISA (Wu et al., 2014) and isolated DNA PCR analysis obtained from the magnetically separated cells (Taban et al., 2009). However, these methods need complex conjugation processes (Wu et al., 2014), pre enrichment of target in the media, and commercial anti Salmonella beads for the sensitive analysis (Taban et al., 2009). In this study, engineered fusion Ab was used for immunomagnetic detection of Salmonella after coupling with two kinds of magnetic beads by ELISA. As it is seen in the graphs, absorbance value showed that Promag beads (Figure 52, A-B) have the capability to capture the target in a low sample volume with high affinity than NEB beads (Figure 52, C). However, applying the NEB beads helps using very low fusion Ab concentration,~ 2 µg/test, while Promag beads need a high concentration, 32 or 110 µg/test, for coupling with them. Results also demonstrated that using a high volume of Promag beads, 80 µl, for coupling with higher amount of fusion Ab increased the absorbance obtained from both target and non target antigens (Figure 52, B). However, the latter increment was not significant statistically since the absorbance obtained from 14028s strain was monitored with significant main effect ($p<0.001$, SPSS inc. One way ANOVA). Thus, using a high volume of beads did not affect the specifity and sensitivity of test result as absorbance value was high enough for the target bacteria. The absence of BG substrate on the beads caused the lack of coupling reaction between the SNAP-Tag on the IgG and magnetic beads. Thus, it was resulted with no capturing the target bacteria in the assay (Figure 52, B). ELISA results performed by NEB beads had also shown the specific recognition of Salmonella, but its absorbance value (Figure 52, C) is lower than Promag beads (Figure 52, A, B) as the fusion Ab concentration was highly lower.

The detection limit of *S. typhimurium* magnetically was reported as $1.2x10^2$cfu/ml (Yang et al., 2013), 10^1 cfu/ml (Wen et al., 2013), 2×10^1 cells (Shim et al., 2014), 10^1 cfu/ml (Taban et al., 2009), 1×10^3 cfu/ml (Wu et al., 2014), and 1×10^3 cfu/ml using aptamer modifed magnetic particles by GNPs based sandwich ELISA (Ronholm et al., 2011). However, they need time consuming requirements such as mice immunization. Moreover, specifically designed systems or advanced equipments, commercial Abs

and further PCR assay to achieve sensitive detection are also needed. In the literature, the combining of the immunomagnetic separation and ELISA was reported with a high number of detected cells, 10^5-10^6 cfu/ml, using 20 µl magnetic beads (Mansfield and Forsythe, 2000). Besides, 143 cells/ml (Afonso et al., 2013) was reported as the detection limit of *S. tyhphimurium* LT2 by electrochemical system. However, not only using the commercial anti-Salmonella magnetic beads but also combining of magnetic field and GNPs technology is needed along with the electrochemical system to achieve this. However, $1.4x10^1$ *S. tyhphimurium* 14028s cells were found as the detection limit by immumagnetic ELISA in this study. This was achieved by engineered fusion Ab which is coupled with 2 µl, 50 µl or 80 µl of different magnetic beads without needed any time consuming or advanced processing systems in 3,5 hr. This result also seemed to be more sensitive than the study reported the detection limit of *S. typhimurum* 14028s as $1x10^3$ cfu/ml by nanoELISA (Ronholm et al., 2011). To the best of our knowledge, *S. typhimurium* detection in a very low cell number, e.g. $1.4x10^1$ cells, spesifically with the generated SNAP-Tagged IgG is the first study in the literature. Therefore, this might be a pioneer for advanced technologies for Salmonella detection.

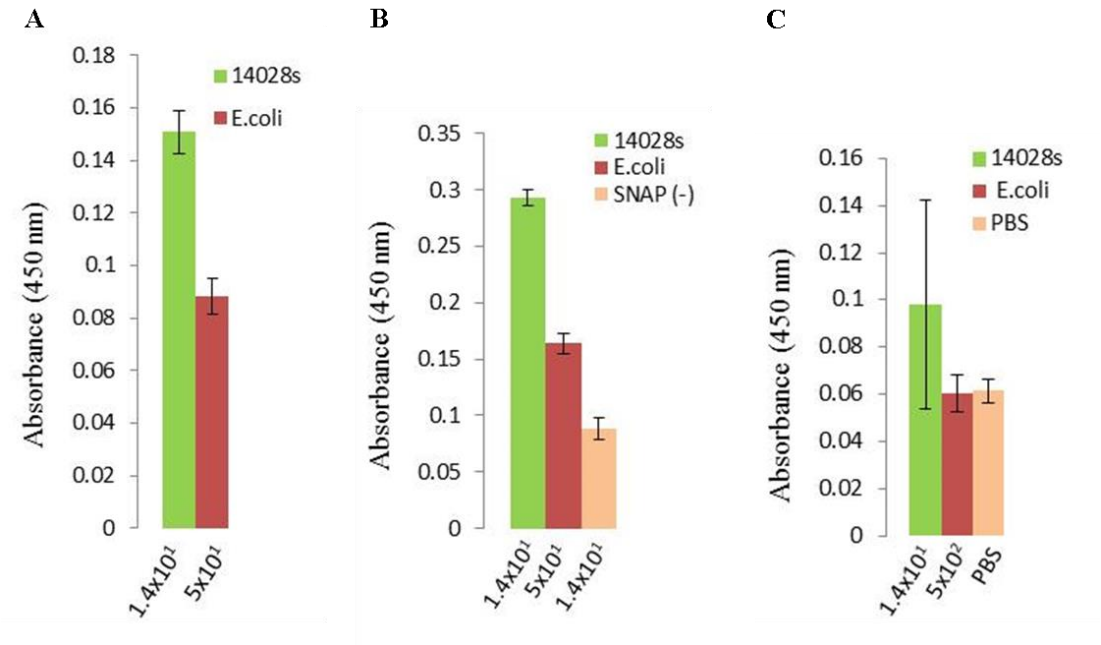
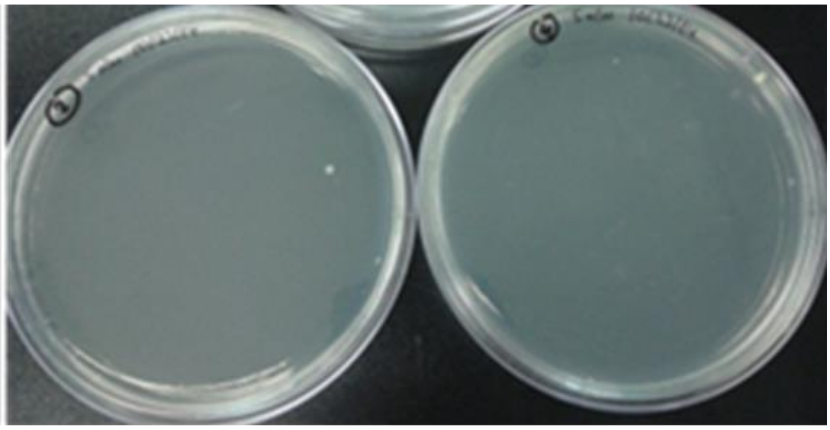


Figure 52. Immunomagnetic ELISA results of Salmonella capture with Promag **(A, B)** and NEB beads **(C)**.~32 µg (couple 1) and ~110 µg (couple 2) fusion Ab was used for coupling with 50 µl and 80 µl Promag beads **(A, B),** respectively. 12.6 µg fusion Ab in 20 µl was used for coupling with 80 µl NEB beads then 2 µl of NEB conjugates (couple 3), e.g. ~0.25 µg fusion Ab was used for ELISA **(C)**. $1.4x10^1$ 14028s cells and $5x10^1$ *E.coli* cells in 20 µl were tested, separately. 14028s: Live *S. typhimurium* 14028s. SNAP (-): No BG substrate was used for magnetic coupling which means that there is no SNAP-Tagged IgG on the beads

In order to make further verification of detected cell numbers, plate counting method was also performed after magnetic separation. It was observed that fusion Abs coupled with 80 µl Promag beads captured almost all of the Salmonella cells while it bound to very low *E.coli* cells. According to plate counting results, 3 colonies of Salmonella were seen, but about 21 colonies were for *E.coli* (Figure 53) after magnetic capture.

S. typhimurium 14028s



E.coli

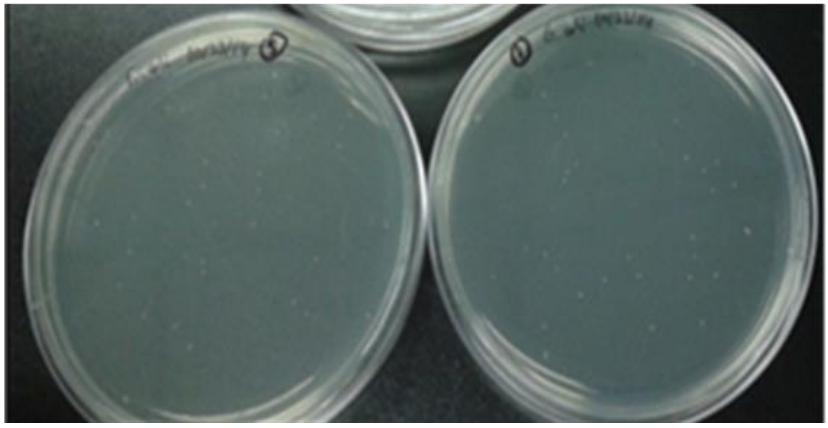


Figure 53. Plate counting of cells after magnetic capturing of Salmonella and *E.coli*

Besides, the number of cells used in immunomagnetic ELISA was counted with hemocytometer and compared the results theoretically. To do this, FK 14028s cells were prepared as desired cell number, which is same with the number of live 14028s cells used in ELISA. Table 20 demonstrates the theoretically numbered and counted cell numbers of 14028s and *E.coli* cells. As it is seen clearly, both numbers are in parallel to each other. Thus, both the graphs shown in Figure 52 and counting methods suggested that the detected cell number is $1.4x10^1$ by immunomagnetic ELISA in a very low sample volume.

Table 20. Comparison of the theoretical and counted cell numbers of *S. typhimurium* 14028s and *E.coli* using hemocytometer (FK: Formalin killed)

(cfu/ml in 20µl)	**FK 14028s**		(cfu/ml in 20µl)	***E.coli***	
	counted	theoretically		counted	theoretically
$7.3x10^2$	11 cells	14 cells	-	-	-
$7.3x10^3$	132 cells	146 cells	$2.5x10^3$	60 cells	50 cells

As a general summary, used methods and detected cell numbers of pathogenic bacteria in this study are also given at Table 21.

Table 21. Summary of used immunogenic methods and detected Salmonella and *E.coli* cells

Target	**Methods**	**Ab**	**Cell number**
S. typhimurium	LF assay	Commercial	$5x10^5$ cells in 100 µl
S. typhimurium (LT2)	LF assay	scFv	$2.8x10^7$ cells in 100 µl
S. typhimurium (LT2)	ELISA	scFv	$2.8x10^4$ cells in 100 µl
S. typhimurium (LT2)	ELISA	Fusion Ab	$4.2x10^8$ cells in 100 µl
S. typhimurium 14028s	Magnetic ELISA	Fusion Ab	$1.4x10^1$ cells in 20 µl
E. coli O157:H7	LF assay	Commercial	$3x10^4$ cells in 100 µl

CHAPTER 4

CONCLUSION

In this research, immunochromatographic test strips were developed by using colloidal GNPs in order to detect the Salmonella and *E.coli* O157:H7. Besides, scFv and humanized full length IgG, fusion Ab, of se155-4 Ab known as specific to Salmonella serogroup B O-polysaccharide antigen were produced and purified. The binding affinity of generated Abs to the different antigenic structures was tested by indirect common ELISA. Detection of Salmonella in a very low cell number was achieved by immunomagnetic ELISA and LF assay platform was also developed by generated scFv.

Developed LF dipstick assays succesfully recognized the Salmonella and *E.coli* O157:H7 cells with a desired number that is enough for triggering the illness. *S. enteritidis* and *S. infantis* were the best recognized bacteria among the tested Salmonella strains. The strips could detect the targets either in the medium or in buffer via sample loading. It can be deduced that the recognition of killed *E.coli* cells by developed test platform showed the applicability of the dispstick assays to the live *E.coli* cells detection in food or water contamination. Therefore, rapid detection of *E.coli* O157:H7 cells will be possible with designed strips in a short time for users. HF180 membrane was decided as the best membrane type for developing the assay for both Salmonella and *E.coli*. 36 nm sized GNPs can be estimated as a perfect size and good label for CSA Abs for the detection of bacteria via strips. Because both the nanoparticles and their conjugates with Abs were found as highly stable after about 8 months at 4˚C. This is crucial for increasing the shelf life of the developed strips.

In this research, production of scFv and fusion Ab was also described. These Abs were found to bind to the LPS of *S. typhimurium* and *S.typhi*, live and formalin killed *S. typhimurium* cells specifically and selectively. Although se155-4 Ab is known as

specific to Salmonella serogroup B in the literature, designed scFv and fusion Ab had also showed high affinity to Salmonella serogroup D in this research. Thus, these Abs can also be a potential for the recognition of *S. typhi* or serogroup D. Among all of the antigens tested in this study, LPS was recognized far superiorly by generated Abs with ELISA. The binding affinity of produced Abs to the antigens was also proportinal to the amount of the Ab used.

To the best of our knowledge, some observations were described as first in this study. These are SNAP-Tagged fusion Ab form of se155-4, binding of produced scFv to the live Salmonella cells and detection of *S. typhimurium* in a very low cell number by immunomagnetic ELISA using fusion Ab without needed extra experimental steps. Thus, those findings can make these Abs a potential candidate for capturing the pathogenic *S. typhimurium* in water or food samples even at low bacterial cell densities. It should be noted that as the generated recombinant Abs have strong sensitivity and selectivity against their antigens, they can also be adopted or used for various Salmonella detecting systems as detecting agent. Since the dipstick format of assays are generally attractive for the end users in terms of their simplicity, cost effectiveness, user friendly application and point of care approach, recognition of Salmonella cells can be advanced with the fusion Abs or scFvs by developed LF strips.

REFERENCES

Ackerson, C. J., Jadzinsky, P. D., Jensen, G. J., & Kornberg, R. D. (2006). Rigid, specific, and discrete gold nanoparticle/antibody conjugates. *Journal of the American Chemical Society*, *128*(8), 2635-2640.

Afonso, A. S., Pérez-López, B., Faria, R. C., Mattoso, L. H., Hernández-Herrero, M., Roig-Sagués, A. X., ... & Merkoçi, A. (2013). Electrochemical detection of Salmonella using gold nanoparticles. *Biosensors and Bioelectronics*, *40*(1), 121-126.

Allos BM, Moore MR, Griffin PM, Tauxe RV. (2004). Surveillance for sporadic foodborne disease in the 21st century: the FoodNet perspective. *Clin Infect Dis* 38(Suppl 3):S115-20.

Alocilja, E. C., Zhang, D., Shi, C. (2013). AuNP-DNA Biosensor for Rapid Detection of Salmonella enterica Serovar Enteritidis. *Advances in Applied Nanotechnology for Agriculture*, *Chapter 3*, 1143, 43–53.

Altekruse, S. F., Cohen, M. L., & Swerdlow, D. L. (1997). Emerging foodborne diseases. *Emerging infectious diseases*, *3*(3), 285.

Altuğ, G. (2011). The Occurrence of Salmonella in Various Marine Environments in Turkey. *SALMONELLA–A DANGEROUS FOODBORNE PATHOGEN*, 73.

Ambrosi, A., Airo, F., & Merkoçi, A. (2009). Enhanced gold nanoparticle based ELISA for a breast cancer biomarker. *Analytical chemistry*, *82*(3), 1151-1156.

Anand, N. N., Dubuc, G., Phipps, J., MacKenzie, C. R., Sadowska, J., Young, N. M., ... & Narang, S. A. (1991a). Synthesis and expression in Escherichia coli of cistronic DNA encoding an antibody fragment specific for a Salmonella serotype B O-antigen. *Gene*, *100*, 39-44.

Anand, N. N., Mandal, S., MacKenzie, C. R., Sadowska, J., Sigurskjold, B., Young, N. M., ... & Narang, S. A. (1991b). Bacterial expression and secretion of various single-chain Fv genes encoding proteins specific for a Salmonella serotype B O-antigen. *Journal of Biological Chemistry*, *266*(32), 21874-21879.

Andersen, D. C., & Reilly, D. E. (2004). Production technologies for monoclonal antibodies and their fragments. *Current Opinion in Biotechnology*, *15*(5), 456-462.

Andrews, W. H., & Hammack, T. S. (2001). Salmonella In: Bacteriological analytical manual, 8th ed. U.S. Department of Agriculture, Washington, D.C.

Ang, G. Y., Yu, C. Y., & Yean, C. Y. (2012). Ambient temperature detection of PCR amplicons with a novel sequence-specific nucleic acid lateral flow biosensor. *Biosensors and Bioelectronics*, *38*(1), 151-156.

Ao, L. M., Gao, F., Pan, B. F., Cui, D. X., & Gu, H. C. (2006). Interaction between gold nanoparticles and bovine serum albumin or sheep antirabbit immunoglobulin G. *Chinese Journal of Chemistry*, *24*(2), 253-256.

Arora, K., Prabhakar, N., Chand, S., & Malhotra, B. D. (2007). E scherichia coli Genosensor Based on Polyaniline. *Analytical Chemistry*, *79*(16), 6152-6158.

Aserkoff, B., Schroeder, S. A., & Brachman, P. S. (1970). Salmonellosis in the United States—a five-year review. *American Journal of Epidemiology*, *92*(1), 13-24.

Aslan, K., Zhang, J., Lakowicz, J. R., & Geddes, C. D. (2004). Saccharide sensing using gold and silver nanoparticles-a review. *Journal of Fluorescence*, *14*(4), 391-400.

Atwell, J. L., Breheney, K. A., Lawrence, L. J., McCoy, A. J., Kortt, A. A., & Hudson, P. J. (1999). scFv multimers of the anti-neuraminidase antibody NC10: length of the linker between VH and VL domains dictates precisely the transition between diabodies and triabodies. *Protein engineering*, *12*(7), 597-604.

Ayong, L. S., Tume, C. B., Wembe, F. E., Simo, G., Asonganyi, T., Lando, G., & Ngu, J. L. (2005). Development and evaluation of an antigen detection dipstick assay for the diagnosis of human onchocerciasis. *Tropical Medicine & International Health*, *10*(3), 228-233.

Backmann, N., Zahnd, C., Huber, F., Bietsch, A., Plückthun, A., Lang, H. P., ... & Gerber, C. (2005). A label-free immunosensor array using single-chain antibody fragments. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(41), 14587-14592.

Baeumner, A. J., Cohen, R. N., Miksic, V., & Min, J. (2003). RNA biosensor for the rapid detection of viable Escherichia coli in drinking water. *Biosensors and Bioelectronics*, *18*(4), 405-413.

Bailey, G. S. (1994). The raising of a polyclonal antiserum to a protein. *Methods Mol. Biol*, *32*, 381-388.

Bayoudh, S., Othmane, A., Ponsonnet, L., & Ouada, H. B. (2008). Electrical detection and characterization of bacterial adhesion using electrochemical impedance spectroscopy-based flow chamber. *Colloids and Surfaces A: physicochemical and engineering aspects*, *318*(1), 291-300.

Bean, N. H., & Griffin, P. M. (1990). Foodborne disease outbreaks in the United States, 1973-1987: pathogens, vehicles, and trends. *Journal of Food Protection®*, *53*(9), 804-817.

Boal, A. K., Ilhan, F., DeRouchey, J. E., Thurn-Albrecht, T., Russell, T. P., & Rotello, V. M. (2000). Self-assembly of nanoparticles into structured spherical and network aggregates. *Nature*, *404*(6779), 746-748.

Boehm, D. A., Gottlieb, P. A., & Hua, S. Z. (2007). On-chip microfluidic biosensor for bacterial detection and identification. *Sensors and Actuators B: Chemical*, *126*(2), 508-514.

Borch, E., Nesbakken, T., & Christensen, H. (1996). Hazard identification in swine slaughter with respect to foodborne bacteria. *International journal of food microbiology*, *30*(1), 9-25.

Boyce, F. M., & Bucher, N. L. (1996). Baculovirus-mediated gene transfer into mammalian cells. *Proceedings of the National Academy of Sciences*, *93*(6), 2348-2352.

Brewer, S. H., Glomm, W. R., Johnson, M. C., Knag, M. K., & Franzen, S. (2005). Probing BSA binding to citrate-coated gold nanoparticles and surfaces. *Langmuir*, *21*(20), 9303-9307.

Brichta, J., Vesela, H., & Franek, M. (2003). Production of scFv recombinant fragments against 2, 4-dichlorophenoxyacetic acid hapten using naive phage library. *Veterinarni Medicina-UZPI (Czech Republic)*. 8(9), 237–247.

Brooks, B. W., Perry, M. B., Lutze-Wallace, C. L., & MacLean, L. L. (2008). Structural characterization and serological specificities of lipopolysaccharides from Salmonella enterica serovar Gallinarum biovar Pullorum standard, intermediate and variant antigenic type strains. *Veterinary microbiology*, *126*(4), 334-344.

Brummell, D. A., Sharma, V. P., Anand, N. N., Bilous, D., Dubuc, G., Michniewicz, J., ... & Sigurskjold, B. W. (1993). Probing the combining site of an anti-carbohydrate antibody by saturation-mutagenesis: role of the heavy-chain CDR3 residues. *Biochemistry*, *32*(4), 1180-1187.

Bundle, D. R., Baumann, H., Brisson, J. R., Gagne, S. M., Zdanov, A., & Cygler, M. (1994a). The solution structure of a trisaccharide-antibody complex: comparison of NMR measurements with a crystal structure. *Biochemistry*, *33*(17), 5183-5192.

Bundle, D. R., Eichler, E., Gidney, M. A. J., Meldal, M., Ragauskas, A., Sigurskjold, B. W., ... & Young, N. M. (1994b). Molecular recognition of a Salmonella trisaccharide epitope by monoclonal antibody Se155-4. *Biochemistry*, *33*(17), 5172-5182.

Burns, S. M., & Hull, S. I. (1998). Comparison of Loss of Serum Resistance by Defined Lipopolysaccharide Mutants and an Acapsular Mutant of UropathogenicEscherichia coli O75: K5. *Infection and immunity*, *66*(9), 4244-4253.

BVL (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit). (1996). Position statement of the ZKBS on classifying Salmonella typhimurium LT2 strains and Salmonella typhimurium strains with stable mutations in aroA, galE or cya and crp genes as recipient organisms in genetic engineering operations. Ref. No.: 6790-10-42.

Cannon, G., & Heinhorst, S. (2010). SERRI Project: Real-Time Detection of Chemicals and Biological Pathogens in Fluids. Detection Of Bacterial Pathogens Using A Portable Quartz Crystal Microbalance (QCM) Based Immunosensor. *Phase Two Progress Report.* SERRI Report 63892-02.

Caruso, F. (2001). Nanoengineering of particle surfaces. *Advanced Materials*, *13*(1), 11-22.

Casals, E., Pfaller, T., Duschl, A., Oostingh, G. J., & Puntes, V. (2010). Time evolution of the nanoparticle protein corona. *ACS nano*, *4*(7), 3623-3632.

Centers for Disease Control and Prevention (CDC. (2005). Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food--10 sites, United States, 2004. *MMWR. Morbidity and mortality weekly report*, *54*(14), 352.

Centers for Disease Control (CDC). CDC estimates of foodborne illness in the United States, 2011.

Chai, Y., Li, S., Horikawa, S., Park, M. K., Vodyanoy, V., & Chin, B. A. (2012). Rapid and sensitive detection of Salmonella Typhimurium on eggshells by using wireless biosensors. *Journal of Food Protection®*, *75*(4), 631-636.

Chalker, R. B., & Blaser, M. J. (1988). A review of human salmonellosis: III. Magnitude of Salmonella infection in the United States. *Review of Infectious Diseases*, *10*(1), 111-124.

Chandler, J., Gurmin, T., & Robinson, N. (2000). The place of gold in rapid tests. *IVD Technology*, *6*(2), 37-49.

Chenggang, S. H. I., Suqing, Z. H. A. O., Zhang, K., Guobao, H. O. N. G., & Zhenyu, Z. H. U. (2008). Preparation of colloidal gold immunochromatography strip for detection of methamidophos residue. *Journal of Environmental Sciences*, *20*(11), 1392-1397.

Choi, D. H., Lee, S. K., Oh, Y. K., Bae, B. W., Lee, S. D., Kim, S., ... & Kim, M. G. (2010). A dual gold nanoparticle conjugate-based lateral flow assay (LFA) method for the analysis of troponin I. *Biosensors and Bioelectronics*, *25*(8), 1999-2002.

Christopher, P., Robinson, N., & Shaw, M. K. (2005). Antibody-label conjugates in lateral-flow assays. In *Drugs of Abuse* (pp. 87-98). Humana Press.

Chu, L., & Robinson, D. K. (2001). Industrial choices for protein production by large-scale cell culture. *Current Opinion in Biotechnology*, *12*(2), 180-187.

Chun, P. (2009). Colloidal gold and other labels for lateral flow immunoassays. In *Lateral Flow Immunoassay* (pp. 1-19). Humana Press.

Clark, L. C., & Lyons, C. (1962). Electrode systems for continuous monitoring in cardiovascular surgery. *Annals of the New York Academy of sciences*, *102*(1), 29-45.

Cogan, T. A., & Humphrey, T. J. (2003). The rise and fall of Salmonella Enteritidis in the UK. *Journal of applied microbiology*, *94*(s1), 114-119.

Colcher, D., Bird, R., Roselli, M., Hardman, K. D., Johnson, S., Pope, S., ... & Schlom, J. (1990). In vivo tumor targeting of a recombinant single-chain antigen-binding protein. *Journal of the National Cancer Institute*, *82*(14), 1191-1197.

Collings, A. F., & Caruso, F. (1997). Biosensors: recent advances. *Reports on Progress in Physics*, *60*(11), 1397-1445

Cudjoe, K. S., Krona, R., Grøn, B., & Olsen, E. (1994). Use of ferrous sulphate and immunomagnetic separation to recover Salmonella enteritidis from raw eggs. *International journal of food microbiology*, *23*(2), 149-158.

Cudjoe, K. S., Hagtvedt, T., & Dainty, R. (1995). Immunomagnetic separation of Salmonella from foods and their detection using immunomagnetic particle (IMP)-ELISA. *International journal of food microbiology*, *27*(1), 11-25.

Cygler, M., Rose, D. R., & Bundle, D. R. (1991). Recognition of a cell-surface oligosaccharide of pathogenic Salmonella by an antibody Fab fragment. *Science*, *253*(5018), 442-445.

D'Aoust, J. Y., Sewell, A. M., Greco, P., Mozola, M. A., & Colvin, R. E. (1995). Performance assessment of the GENE-TRAK® colorimetric probe assay for the detection of foodborne Salmonella spp. *Journal of Food Protection®*,*58*(10), 1069-1076.

Daniel, M. C., & Astruc, D. (2004). Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *Chemical reviews*, *104*(1), 293-346.

DeCory, T. R., Durst, R. A., Zimmerman, S. J., Garringer, L. A., Paluca, G., DeCory, H. H., & Montagna, R. A. (2005). Development of an immunomagnetic bead-immunoliposome fluorescence assay for rapid detection of Escherichia coli O157: H7 in aqueous samples and comparison of the assay with a standard microbiological method. *Applied and environmental microbiology*, *71*(4), 1856-1864.

Delmulle, B. S., De Saeger, S. M., Sibanda, L., Barna-Vetro, I., & Van Peteghem, C. H. (2005). Development of an immunoassay-based lateral flow dipstick for the rapid detection of aflatoxin B1 in pig feed. *Journal of agricultural and food chemistry*, *53*(9), 3364-3368.

Deng, S. J., MacKenzie, C. R., Sadowska, J., Michniewicz, J., Young, N. M., Bundle, D. R., & Narang, S. A. (1994). Selection of antibody single-chain variable fragments with improved carbohydrate binding by phage display. *Journal of biological chemistry*, *269*(13), 9533-9538.

Deng, S. J., MacKenzie, C. R., Hirama, T., Brousseau, R., Lowary, T. L., Young, N. M., ... & Narang, S. A. (1995). Basis for selection of improved carbohydrate-binding single-chain antibodies from synthetic gene libraries. *Proceedings of the National Academy of Sciences*, *92*(11), 4992-4996.

Deng, Y. P., Zhao, H. Q., & Jiang, L. (2000). Applications of nanogold particles in biomimetic engineering. *China Basic Sci*, *9*, 11-17.

Derjaguin, B. V., & Landau, L. (1941). Theory of the stability of strongly charged lyophobic sols and of the adhesion of strongly charged particles in solutions of electrolytes. *Acta physicochim. URSS*, *14*(6), 633-662.

Di Fabio, J. L., Brisson, J. R., & Perry, M. B. (1989). Structure of the lipopolysaccharide antigenic O-chain produced by Salmonella ohio (0: 6, 7).*Carbohydrate research*, *189*, 161-168.

Dolezal, O., De Gori, R., Walter, M., Doughty, L., Hattarki, M., Hudson, P. J., & Kortt, A. A. (2003). Single-chain Fv multimers of the anti-neuraminidase antibody NC10: the residue at position 15 in the VL domain of the scFv-0 (VL− VH) molecule is primarily responsible for formation of a tetramer–trimer equilibrium. *Protein engineering*, *16*(1), 47-56.

Dominguez-Medina, S., McDonough, S., Swanglap, P., Landes, C. F., & Link, S. (2012). In situ measurement of bovine serum albumin interaction with gold nanospheres. *Langmuir*, *28*(24), 9131-9139.

Dorman, V., Aslan, S., Gülsün, S., Kubat, N. K., Çevrim, U., Yaşar, E., ... & Öncül, A. (2011). Two Consecutive Outbreaks of Foodborne Gastroenteritis Caused by Salmonella Enteritidis in Turkey. *Turkiye Klinikleri Journal of Medical Sciences*, *31*(4), 935.

Dorn, C. R., & Angrick, E. J. (1991). Serotype O157: H7 Escherichia coli from bovine and meat sources. *Journal of clinical microbiology*, *29*(6), 1225-1231.

Droz, E., Taborelli, M., Descouts, P., Wells, T. N., & Werlen, R. C. (1996). Covalent immobilization of immunoglobulins G and Fab′ fragments on gold substrates for scanning force microscopy imaging in liquids. *Journal of Vacuum Science & Technology B*, *14*(2), 1422-1426.

D'Souza, D. H., Critzer, F. J., & Golden, D. A. (2009). Real-time reverse-transcriptase polymerase chain reaction for the rapid detection of Salmonella using inv A primers. *Foodborne pathogens and disease*, *6*(9), 1097-1106.

Dwivedi, H. P., & Jaykus, L. A. (2011). Detection of pathogens in foods: the current state-of-the-art and future directions. *Critical reviews in microbiology*,*37*(1), 40-63.

Dykman, L. A., Bogatyrev, V. A., Khlebtsov, B. N., & Khlebtsov, N. G. (2005). A protein assay based on colloidal gold conjugates with trypsin. *Analytical biochemistry*, *341*(1), 16-21.

Dykman, L. A., Bogatyrev, V. A., Shchegolev, S. Y., & Khlebtsov, N. G. (2008). Gold Nanoparticles: Synthesis, Properties, and Biomedical Applications. *Science, Moscow*.

Ekborg, G., Eklind, K., Garegg, P. J., Gotthammar, B., Carlsson, H. E., Lindberg, A. A., & Svenungsson, B. (1977). Artificial diaccharide-protein conjugates as immunogens for the preparation of specific anti-salmonella O-antisera. *Immunochemistry*, *14*(2), 153-157.

Englebienne P. (2000). Immune and Receptor Assays in Theory and Practice, *CRC Press*, New York, pp. 233–234.

Eurosurveillance Editorial Team. (2012). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2010. *Euro surveillance: bulletin Européen sur les maladies transmissibles= European communicable disease bulletin*, *17*(10), 2597, p. 442, 2012.

Fang, C., Chen, Z., Li, L., & Xia, J. (2011). Barcode lateral flow immunochromatographic strip for prostate acid phosphatase determination. *Journal of pharmaceutical and biomedical analysis*, *56*(5), 1035-1040.

Fluit, A. C., Widjojoatmodjo, M. N., Box, A. T., Torensma, R., & Verhoef, J. (1993). Rapid detection of salmonellae in poultry with the magnetic immuno-polymerase chain reaction assay. *Applied and environmental microbiology*,*59*(5), 1342-1346.

Frens, G. (1973). Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions. *Nature*, *241*(105), 20-22.

Freudenberg, M. A., Fomsgaard, A., Mitov, I., & Galanos, C. (1989). ELISA for antibodies to lipid A, lipopolysaccharides and other hydrophobic antigens. *Infection*, *17*(5), 322-328.

Fu, Z., Rogelj, S., & Kieft, T. L. (2005). Rapid detection of Escherichia coli O157: H7 by immunomagnetic separation and real-time PCR. *International journal of food microbiology*, *99*(1), 47-57.

Fuchs, M., Kämpfer, S., Helmsing, S., Spallek, R., Oehlmann, W., Prilop, W., ... & Hust, M. (2014). Novel human recombinant antibodies against Mycobacterium tuberculosis antigen 85B. *BMC biotechnology*, *14*(1), 68.

Gamella, M., Campuzano, S., Parrado, C., Reviejo, A. J., & Pingarrón, J. M. (2009). Microorganisms recognition and quantification by lectin adsorptive affinity impedance. *Talanta*, *78*(4), 1303-1309.

Gandhi, M., & Chikindas, M. L. (2007). Listeria: a foodborne pathogen that knows how to survive. *International journal of food microbiology*, *113*(1), 1-15.

Gehring, A. G., Albin, D. M., Bhunia, A. K., Reed, S. A., Tu, S. I., & Uknalis, J. (2006). Antibody Microarray Detection of Escherichia c oli O157: H7: Quantification, Assay Limitations, and Capture Efficiency. *Analytical chemistry*, *78*(18), 6601-6607.

Geoghegan, W. D. (1988). The effect of three variables on adsorption of rabbit IgG to colloidal gold. *Journal of Histochemistry & Cytochemistry*, *36*(4), 401-407.

Gervais, L., Gel, M., Allain, B., Tolba, M., Brovko, L., Zourob, M., ... & Evoy, S. (2007). Immobilization of biotinylated bacteriophages on biosensor surfaces. *Sensors and Actuators B: Chemical*, *125*(2), 615-621.

Ghosh, S. S., Kao, P. M., McCue, A. W., & Chappelle, H. L. (1990). Use of maleimide-thiol coupling chemistry for efficient syntheses of oligonucleotide-enzyme conjugate hybridization probes. *Bioconjugate chemistry*, *1*(1), 71-76.

Gibson, D. G. (2011). Enzymatic assembly of overlapping DNA fragments. *Methods Enzymol*, *498*, 349-361.

Goncagül, G., Günaydin, E., & Carli, K. T. (2005). Prevalence of Salmonella serogroups in chicken meat. *Turkish Journal of Veterinary and Animal Sciences*, *29*(1), 103-106.

Gonzalez, J. M., Foley, M. W., Bieber, N. M., Bourdelle, P. A., & Niedbala, R. S. (2011). Development of an ultrasensitive immunochromatography test to detect nicotine metabolites in oral fluids. *Analytical and bioanalytical chemistry*, *400*(10), 3655-3664.

Gordon, E., Horsefield, R., Swarts, H. G., de Pont, J. J. H., Neutze, R., & Snijder, A. (2008). Effective high-throughput overproduction of membrane proteins in Escherichia coli. *Protein expression and purification*, *62*(1), 1-8.

Gottschamel, J., Richter, L., Mak, A., Jungreuthmayer, C., Birnbaumer, G., Milnera, M., ... & Ertl, P. (2009). Development of a disposable microfluidic biochip for multiparameter cell population measurements. *Analytical chemistry*, *81*(20), 8503-8512.

Griffin, T. J., Seth, G., Xie, H., Bandhakavi, S., & Hu, W. S. (2007). Advancing mammalian cell culture engineering using genome-scale technologies. *Trends in biotechnology*, *25*(9), 401-408.

Gronemeyer, T., Chidley, C., Juillerat, A., Heinis, C., & Johnsson, K. (2006). Directed evolution of O6-alkylguanine-DNA alkyltransferase for applications in protein labeling. *Protein Engineering Design and Selection*, *19*(7), 309-316.

Grzelczak, M., Pérez-Juste, J., Mulvaney, P., & Liz-Marzán, L. M. (2008). Shape control in gold nanoparticle synthesis. *Chemical Society Reviews*, *37*(9), 1783-1791.

Guo, Y. R., Liu, S. Y., Gui, W. J., & Zhu, G. N. (2009). Gold immunochromatographic assay for simultaneous detection of carbofuran and triazophos in water samples. *Analytical biochemistry*, *389*(1), 32-39.

Hagens, S., & Loessner, M. J. (2007). Application of bacteriophages for detection and control of foodborne pathogens. *Applied Microbiology and Biotechnology*, *76*(3), 513-519.

Haiss, W., Thanh, N. T., Aveyard, J., & Fernig, D. G. (2007). Determination of size and concentration of gold nanoparticles from UV-vis spectra. *Analytical chemistry*, *79*(11), 4215-4221.

Hargrett-Bean, N. T., Pavia, A. T., & Tauxe, R. V. (1988). Salmonella isolates from humans in the United States, 1984-1986. *MMWR. CDC surveillance summaries: Morbidity and mortality weekly report. CDC surveillance summaries/Centers for Disease Control*, *37*(2), 25-31.

Hayat, M A. (1989). Colloidal Gold: Principles, Methods, and Applications; *Academic Press: New York*, Vol. 1.

He, Y., Zhang, S., Zhang, X., Baloda, M., Gurung, A. S., Xu, H., ... & Liu, G. (2011). Ultrasensitive nucleic acid biosensor based on enzyme–gold nanoparticle dual label and lateral flow strip biosensor. *Biosensors and Bioelectronics*, *26*(5), 2018-2024.

Hellerqvist, C. G., Lindberg, B., Svensson, S., Holme, T., & Lindberg, A. A. (1968). Structural studies on the O-specific side-chains of the cell-wall lipopolysaccharide from Salmonella typhimurium 395 MS. *Carbohydrate Research*, *8*(1), 43-55.

Hellerqvıst, C. G. (1972). Sequence Analysis of the Polysaccharides from Salmonella. *Acta Chemica Scandinavica*, *26*(8), 3282-3286.

Heiman, K. E., Mody, R. K., Johnson, S. D., Griffin, P. M., & Gould, L. H. (2015). Escherichia coli O157 Outbreaks in the United States, 2003–2012.*Emerging infectious diseases*, *21*(8), 1293.

Hink, M. A., Griep, R. A., Borst, J. W., van Hoek, A., Eppink, M. H., Schots, A., & Visser, A. J. (2000). Structural dynamics of green fluorescent protein alone and fused with a single chain Fv protein. *Journal of Biological Chemistry*, *275*(23), 17556-17560.

Holliger, P., & Hudson, P. J. (2005). Engineered antibody fragments and the rise of single domains. *Nature biotechnology*, *23*(9), 1126-1136.

Hoorfar, J. (2011). Rapid detection, characterization, and enumeration of foodborne pathogens. *APMIS*, *119*(s133), 1-24.

Horisberger, M., & Rosset, J. (1977). Colloidal gold, a useful marker for transmission and scanning electron microscopy. *Journal of Histochemistry & Cytochemistry*, *25*(4), 295-305.

Hossain, S. Z., Ozimok, C., Sicard, C., Aguirre, S. D., Ali, M. M., Li, Y., & Brennan, J. D. (2012). Multiplexed paper test strip for quantitative bacterial detection. *Analytical and bioanalytical chemistry*, *403*(6), 1567-1576.

Hostetler, M. J., Wingate, J. E., Zhong, C. J., Harris, J. E., Vachet, R. W., Clark, M. R., ... & Glish, G. L. (1998). Alkanethiolate gold cluster molecules with core diameters from 1.5 to 5.2 nm: core and monolayer properties as a function of core size. *Langmuir*, *14*(1), 17-30.

Hudson, P. J., & Kortt, A. A. (1999). High avidity scFv multimers; diabodies and triabodies. *Journal of immunological methods*, *231*(1), 177-189.

Huh, S. H., Do, H. J., Lim, H. Y., Kim, D. K., Choi, S. J., Song, H., ... & Kim, J. H. (2007). Optimization of 25kDa linear polyethylenimine for efficient gene delivery. *Biologicals*, *35*(3), 165-171.

Hunter, R. J. (2001). *Foundations of colloid science*. Oxford University Press.

Huo, T.M., Peng, C. F., Xu, C. L., Liu, L. Q. (2006). Development of colloidalgold-based immunochromatographic assay for the rapid detection of medroxyprogesterone acetate residues. *Food Agric. Immunol.*, 17:183-190.

Hygiena. MicroSnap Logo, E. coli. http://www.hygiena.com/microsnap-e-coli-other.html last access date: 08.03.2016.

Inal, T., Bese, M., Ugur, M., Tantas, A. (1979). Izmir seaport waters and Salmonella contamination. Research Bulletin TUBITAK, Ankara 293, 20.

Ince, O. T., Yalçin, S. S., Yurdakök, K., Özmert, E. N., Aydin, A., Baris, Z., & Gür, D. (2012). Salmonella gastroenteritis in children (clinical characteristics and antibiotic susceptibility): comparison of the years 1995-2001 and 2002-2008. *The Turkish journal of pediatrics*, *54*(5), 465.

Ijeh, M. (2011). *Covalent gold nanoparticle—antibody conjugates for sensitivity improvement in LFIA* (Doctoral dissertation, Hamburg University).

Jäger, V., Büssow, K., Wagner, A., Weber, S., Hust, M., Frenzel, A., & Schirrmann, T. (2013). High level transient production of recombinant antibodies and antibody fusion proteins in HEK293 cells. *BMC biotechnology*, *13*(1), 52.

Jain, P. K., Lee, K. S., El-Sayed, I. H., & El-Sayed, M. A. (2006). Calculated absorption and scattering properties of gold nanoparticles of different size, shape, and composition: applications in biological imaging and biomedicine. *The Journal of Physical Chemistry B*, *110*(14), 7238-7248.

Jalah, R., Rosati, M., Kulkarni, V., Patel, V., Bergamaschi, C., Valentin, A., ... & Pavlakis, G. N. (2007). Efficient systemic expression of bioactive IL-15 in mice upon delivery of optimized DNA expression plasmids. *DNA and cell biology*, *26*(12), 827-840.

Jann K,, Westphal, O. (1975). Microbial polysaccharides. In: Sela M (ed) The antigens, vol III. *Academic Press, New York*, pp 1–125.

Jaradat, Z. W., & Zawistowski, J. (1996). Production and characterization of monoclonal antibodies against the O-5 antigen of Salmonella typhimurium lipopolysaccharide. *Applied and environmental microbiology*, *62*(1), 1-5.

Jiang, X. (2009). Gold nanoparticles for biosensing. *Doctoral dissertation*, Institute of Fundamental Science, Massey University, New Zeland

Johne, B., Hansen, K., Mørk, E., & Holtlund, J. (1995). Colloidal gold conjugated monoclonal antibodies, studied in the BIAcore biosensor and in the Nycocard immunoassay format. *Journal of immunological methods*, *183*(1), 167-174.

Jung, B. Y., Jung, S. C., & Kweon, C. H. (2005). Development of a rapid immunochromatographic strip for detection of Escherichia coli O157. *Journal of Food Protection®*, *68*(10), 2140-2143.

Käferstein FK, Motarjemi Y, Bettcher DW. (1997). Foodborne disease control: a transnational challenge. Emerging Infect. Dis. 3, pp. 503-510

Kalorama Information. (2006). The Worldwide Market for In Vitro Diagnostic Tests, 5th Edition April, Retrieved from www.kaloramainformation.com. 09.03.2016.

Karsunke, X. Y., Wang, H., Weber, E., McLean, M. D., Niessner, R., Hall, J. C., & Knopp, D. (2012). Development of single-chain variable fragment (scFv) antibodies against hapten benzo [a] pyrene: a binding study. *Analytical and bioanalytical chemistry*, *402*(1), 499-507.

Kawasaki, T., Gouda, M. D., Sawasaki, T., Takai, K., & Endo, Y. (2003). Efficient synthesis of a disulfide-containing protein through a batch cell-free system from wheat germ. *European Journal of Biochemistry*, *270*(23), 4780-4786.

Kerman, K., Kobayashi, M., & Tamiya, E. (2004). Recent trends in electrochemical DNA biosensor technology. *Measurement Science and Technology*, *15*(2), R1.

Kerr, P., Chart, H., Finlay, D., Pollock, D. A., MacKie, D. P., & Ball, H. J. (2001). Development of a monoclonal sandwich ELISA for the detection of animal and human Escherichia coli O157 strains. *Journal of applied microbiology*, *90*(4), 543-549.

Khan, K. H. (2013). Gene expression in mammalian cells and its applications. *Adv Pharm Bull*, *3*(2), 257-63.

Kim, S. (2010). Salmonella serovars from foodborne and waterborne diseases in Korea, 1998-2007: total isolates decreasing versus rare serovars emerging.*Journal of Korean medical science*, *25*(12), 1693-1699.

Kim, G., Park, S. B., Moon, J. H., & Lee, S. (2013). Detection of pathogenic Salmonella with nanobiosensors. *Analytical Methods*, *5*(20), 5717-5723.

Kim, G., Moon, J. H., Moh, C. Y., & Lim, J. G. (2015). A microfluidic nano-biosensor for the detection of pathogenic Salmonella. *Biosensors and Bioelectronics*, *67*, 243-247.

Kim, S., Frye, J. G., Hu, J., Fedorka-Cray, P. J., Gautom, R., & Boyle, D. S. (2006). Multiplex PCR-based method for identification of common clinical serotypes of Salmonella enterica subsp. enterica. *Journal of clinical microbiology*, *44*(10), 3608-3615.

Kimling, J., Maier, M., Okenve, B., Kotaidis, V., Ballot, H., & Plech, A. (2006). Turkevich method for gold nanoparticle synthesis revisited. *The Journal of Physical Chemistry B*, *110*(32), 15700-15707.

Knight, J. B., Halperin, S. A., West, K. A., & Lee, S. F. (2008). Expression of a functional single-chain variable-fragment antibody against complement receptor 1 in Streptococcus gordonii. *Clinical and Vaccine Immunology*, *15*(6), 925-931.

Kricka, L. J. (1998). Prospects for chemiluminescent and bioluminescent immunoassay and nucleic acid assays in food testing and the pharmaceutical industry. *Journal of bioluminescence and chemiluminescence*, *13*(4), 189-193.

Kohn, J. (1968). An immunochromatographic technique. *Immunology*, *15*(6), 863.

Köhler, G., & Milstein, C. (1975). Continuous cultures of fused cells secreting antibody of predefined specificity. *nature*, *256*, 495-497.

Kortt, A. A., Malby, R. L., Caldwell, J. B., Gruen, L. C., Ivancic, N., Lawrence, M. C., ... & Colman, P. M. (1994). Recombinant antineuraminidase single chain Fv antibody: characterization, formation of dimer and higher molecular mass multimers and the solution of the crystal structure of the scFv-neuraminidase complex. *Eur. J. Biochem*, *221*, 151-157.

Krauss, J., Arndt, M. A. E., Zhu, Z., Newton, D. L., Vu, B. K., Choudhry, V., ... & Richards, J. (2004). Impact of antibody framework residue VH-71 on the stability of a humanised anti-MUC1 scFv and derived immunoenzyme. *British journal of cancer*, *90*(9), 1863-1870.

Krysinski, E. P., & Heimsch, R. C. (1977). Use of enzyme-labeled antibodies to detect Salmonella in foods. *Applied and environmental microbiology*, *33*(4), 947-954.

Kumar, S., Balakrishna, K., & Batra, H. V. (2008). Enrichment-ELISA for detection of Salmonella typhi from food and water samples. *Biomedical and environmental sciences*, *21*(2), 137-143.

Kuyper, C. L., Fujimoto, B. S., Zhao, Y., Schiro, P. G., & Chiu, D. T. (2006). Accurate sizing of nanoparticles using confocal correlation spectroscopy. *The Journal of Physical Chemistry B*, *110*(48), 24433-24441.

Laderman, E. I., Whitworth, E., Dumaual, E., Jones, M., Hudak, A., Hogrefe, W., ... & Groen, J. (2008). Rapid, sensitive, and specific lateral-flow immunochromatographic point-of-care device for detection of herpes simplex virus type 2-specific immunoglobulin G antibodies in serum and whole blood. *Clinical and Vaccine Immunology*, *15*(1), 159-163.

Laura, A., Claudio, B., Cristina, G., & Gianfranco, G. (2011). A lateral flow immunoassay for measuring ochratoxin A: development of a single system for maize, wheat and durum wheat. *Food Control*, *22*(12), 1965-1970.

Le Gall, F., Bové, J. M., & Garnier, M. (1998). Engineering of a single-chain variable-fragment (scFv) antibody specific for the stolbur phytoplasma (Mollicute) and its expression in Escherichia coli and tobacco plants. *Applied and environmental microbiology*, *64*(11), 4566-4572.

Lenggoro, I. W., Xia, B., Okuyama, K., & de la Mora, J. F. (2002). Sizing of colloidal nanoparticles by electrospray and differential mobility analyzer methods. *Langmuir*, *18*(12), 4584-4591.

Leuvering, J. H., Thal, P. J., Van der Waart, M., & Schuurs, A. H. (1981). A sol particle agglutination assay for human chorionic gonadotrophin. *Journal of immunological methods*, *45*(2), 183-194.

Levy, B. S. (1974). The economic impact of a food-borne salmonellosis outbreak. *JAMA*, *230*(9), 1281-1282.

Li, Y., Cheng, P., Gong, J., Fang, L., Deng, J., Liang, W., & Zheng, J. (2012). Amperometric immunosensor for the detection of Escherichia coli O157: H7 in food specimens. *Analytical biochemistry*, *421*(1), 227-233.

Liébana, S., Lermo, A., Campoy, S., Barbé, J., Alegret, S., & Pividori, M. I. (2009). Magneto immunoseparation of pathogenic bacteria and electrochemical magneto genosensing of the double-tagged amplicon. *Analytical chemistry*,*81*(14), 5812-5820.

Lin, Y. H., Chen, S. H., Chuang, Y. C., Lu, Y. C., Shen, T. Y., Chang, C. A., & Lin, C. S. (2008). Disposable amperometric immunosensing strips fabricated by Au nanoparticles-modified screen-printed carbon electrodes for the detection of foodborne pathogen Escherichia coli O157: H7. *Biosensors and Bioelectronics*, *23*(12), 1832-1837.

Link, S., & El-Sayed, M. A. (1999). Size and temperature dependence of the plasmon absorption of colloidal gold nanoparticles. *The Journal of Physical Chemistry B*, *103*(21), 4212-4217.

Linscott, D. W. (2008). *Linscott's Directory of Immunological and Biological Reagents*.

Liu, Y., & Li, Y. (2002). Detection of Escherichia coli O157: H7 using immunomagnetic separation and absorbance measurement. *Journal of microbiological methods*, *51*(3), 369-377.

Liu, J., Mazumdar, D., & Lu, Y. (2006). A Simple and Sensitive "Dipstick" Test in Serum Based on Lateral Flow Separation of Aptamer-Linked Nanostructures. *Angew. Chem. Int. Ed*. 45, 7955 –7959.

Liu, Y. S., Walter, T. M., Chang, W. J., Lim, K. S., Yang, L., Lee, S. W., ... & Bashir, R. (2007). Electrical detection of germination of viable model Bacillus anthracis spores in microfluidic biochips. *Lab on a Chip*, *7*(5), 603-610.

Liu, Y., Liu, Y., Mernaugh, R. L., & Zeng, X. (2009). Single chain fragment variable recombinant antibody functionalized gold nanoparticles for a highly sensitive colorimetric immunoassay. *Biosensors and Bioelectronics*, *24*(9), 2853-2857.

Liu, B., Perepelov, A. V., Li, D., Sof'ya, N. S., Han, Y., Shashkov, A. S., ... & Wang, L. (2010). Structure of the O-antigen of Salmonella O66 and the genetic basis for similarity and differences between the closely related O-antigens of Escherichia coli O166 and Salmonella O66. *Microbiology*, *156*(6), 1642-1649.

Liu, C. C., Yeung, C. Y., Chen, P. H., Yeh, M. K., & Hou, S. Y. (2013). Salmonella detection using 16S ribosomal DNA/RNA probe-gold nanoparticles and lateral flow immunoassay. *Food chemistry*, *141*(3), 2526-2532.

Lizano, S., Ramanathan, S., Feltus, A., Witkowski, A., & Daunert, S. (1997). Bioluminescence competitive binding assays for biotin based on photoprotein aequorin. *Methods in enzymology*, *279*, 296-303

Liz-Marzán, L. M. (2006). Tailoring surface plasmons through the morphology and assembly of metal nanoparticles. *Langmuir*, *22*(1), 32-41.

Lou, S., Ye, J. Y., Li, K. Q., & Wu, A. (2012). A gold nanoparticle-based immunochromatographic assay: The influence of nanoparticulate size. *Analyst*, *137*(5), 1174-1181.

Lui, C., Cady, N. C., & Batt, C. A. (2009). Nucleic acid-based detection of bacterial pathogens using integrated microfluidic platform systems. *Sensors*, *9*(5), 3713-3744.

Luo, D., Geng, M., Schultes, B., Ma, J., Xu, D. Z., Hamza, N., ... & Madiyalakan, R. (1998). Expression of a fusion protein of scFv–biotin mimetic peptide for immunoassay. *Journal of biotechnology*, *65*(2), 225-228.

Lüderitz, O., Staub, A. M., & Westphal, O. (1966). Immunochemistry of O and R antigens of Salmonella and related Enterobacteriaceae. *Bacteriological Reviews*, *30*(1), 192.

Lynch, M. J., Leon-Velarde, C. G., McEwen, S., & Odumeru, J. A. (2004). Evaluation of an automated immunomagnetic separation method for the rapid detection of Salmonella species in poultry environmental samples. *Journal of microbiological methods*, *58*(2), 285-288.

Ma, X., Jiang, Y., Jia, F., Yu, Y., Chen, J., & Wang, Z. (2014). An aptamer-based electrochemical biosensor for the detection of Salmonella. *Journal of microbiological methods*, *98*, 94-98.

MacKenzie, C. R., Hirama, T., Deng, S. J., Bundle, D. R., Narang, S. A., & Young, N. M. (1996). Analysis by surface plasmon resonance of the influence of valence on the ligand binding affinity and kinetics of an anti-carbohydrate antibody. *Journal of Biological Chemistry*, *271*(3), 1527-1533.

Maciorowski, K. G., Peña, J., Pillai, S. D., & Ricke, S. C. (1998). Application of gene amplification in conjunction with a hybridization sensor for rapid detection of Salmonella spp. and fecal contamination indicators in animal feeds. *Journal of Rapid Methods & Automation in Microbiology*, *6*(3), 225-238.

Mairhofer, J., Roppert, K., & Ertl, P. (2009). Microfluidic systems for pathogen sensing: a review. *Sensors*, *9*(6), 4804-4823.

Makvandi-Nejad, S., McLean, M. D., Hirama, T., Almquist, K. C., MacKenzie, C. R., & Hall, J. C. (2005). Transgenic tobacco plants expressing a dimeric single-chain variable fragment (scFv) antibody against Salmonella enterica serotype Paratyphi B. *Transgenic research*, *14*(5), 785-792.

Malorny, B., Paccassoni, E., Fach, P., Bunge, C., Martin, A., & Helmuth, R. (2004). Diagnostic real-time PCR for detection of Salmonella in food. *Applied and environmental microbiology*, *70*(12), 7046-7052.

Mansfield, L. P., & Forsythe, S. J. (2000). The detection of Salmonella using a combined immunomagnetic separation and ELISA end-detection procedure. *Letters in applied microbiology*, *31*(4), 279-283.

Manzano, M., Cocolin, L., Astori, G., Pipan, C., Botta, G. A., Cantoni, C., & Comi, G. (1998). Development of a PCR microplate-capture hybridization method for simple, fast and sensitive detection ofSalmonellaserovars in food.*Molecular and cellular probes*, *12*(4), 227-234.

Mao, X., Ma, Y., Zhang, A., Zhang, L., Zeng, L., & Liu, G. (2009). Disposable nucleic acid biosensors based on gold nanoparticle probes and lateral flow strip. *Analytical chemistry*, *81*(4), 1660-1668.

Matveev, S. V., Lewis, J. C., & Daunert, S. (1999). Genetically engineered obelin as a bioluminescent label in an assay for a peptide. *Analytical biochemistry*, *270*(1), 69-74.

Mayer, A. B., & Mark, J. E. (1998). Colloidal gold nanoparticles protected by water-soluble homopolymers and random copolymers. *European polymer journal*, *34*(1), 103-108.

Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., ... & Tauxe, R. V. (1999). Food-related illness and death in the United States.*Emerging infectious diseases*, *5*(5), 607.

Melton, D. W., Ketchen, A. M., Kind, A. J., McEwan, C., Paisley, D., & Selfridge, J. (2001). A one-step gene amplification system for use in cultured mammalian cells and transgenic animals. *Transgenic research*, *10*(2), 133-142.

Mernier, G., Hasenkamp, W., Piacentini, N., & Renaud, P. (2010). Multiple-frequency impedance measurements in continuous flow for the evaluation of electrical lysis of yeast cells. *Procedia Engineering*, *5*, 37-40.

Mettler, M., Grimm, F., Capelli, G., Camp, H., & Deplazes, P. (2005). Evaluation of enzyme-linked immunosorbent assays, an immunofluorescent-antibody test, and two rapid tests (immunochromatographic-dipstick and gel tests) for serological diagnosis of symptomatic and asymptomatic Leishmania infections in dogs. *Journal of clinical microbiology*, *43*(11), 5515-5519.

Michael, N. P., Chester, K. A., Melton, R. G., Robson, L., Nicholas, W., Boden, J. A., ... & Minton, N. P. (1996). In vitro and in vivo characterisation of a recombinant carboxypeptidase G 2:: anti-CEA scFv fusion protein.*Immunotechnology*, *2*(1), 47-57.

Miethe, S., Meyer, T., Wöhl-Bruhn, S., Frenzel, A., Schirrmann, T., Dübel, S., & Hust, M. (2013). Production of single chain fragment variable (scFv) antibodies in Escherichia coli using the LEX™ bioreactor. *Journal of biotechnology*, *163*(2), 105-111.

Mikawa, A. Y., Santos, S. A. T., Kenfe, F. R., da Silva, F. H., & da Costa, P. I. (2009). Development of a rapid one-step immunochromatographic assay for HCV core antigen detection. *Journal of virological methods*, *158*(1), 160-164.

Mohan, C., Kim, Y. G., Koo, J., & Lee, G. M. (2008). Assessment of cell engineering strategies for improved therapeutic protein production in CHO cells. *Biotechnology Journal*, *3*(5), 624-630.

Molinelli, A., Grossalber, K., & Krska, R. (2009). A rapid lateral flow test for the determination of total type B fumonisins in maize. *Analytical and bioanalytical chemistry*, *395*(5), 1309-1316.

Moongkarndi, P., Rodpai, E., & Kanarat, S. (2011). Evaluation of an immunochromatographic assay for rapid detection of Salmonella enterica serovars Typhimurium and Enteritidis. *Journal of Veterinary Diagnostic Investigation*, *23*(4), 797-801.

Muhammad-Tahir, Z., & Alocilja, E. C. (2003). A conductometric biosensor for biosecurity. *Biosensors and Bioelectronics*, *18*(5), 813-819.

Murphy, C., Carroll, C., & Jordan, K. N. (2006). Environmental survival mechanisms of the foodborne pathogen Campylobacter jejuni. *Journal of Applied Microbiology*, *100*(4), 623-632.

Nara, S., Tripathi, V., Singh, H., & Shrivastav, T. G. (2010). Colloidal gold probe based rapid immunochromatographic strip assay for cortisol. *Analytica chimica acta*, *682*(1), 66-71.

Narayanan, R., & El-Sayed, M. A. (2005). Catalysis with transition metal nanoparticles in colloidal solution: nanoparticle shape dependence and stability. *The Journal of Physical Chemistry B*, *109*(26), 12663-12676.

Nedelman, M. A., Shealy, D. J., Boulin, R., Brunt, E., Seasholtz, J. I., Allen, I. E., ... & Pang, R. H. (1993). Rapid infarct imaging with a technetium-99m-labeled antimyosin recombinant single-chain Fv: evaluation in a canine model of acute myocardial infarction. *Journal of nuclear medicine: official publication, Society of Nuclear Medicine*, *34*(2), 234-241.

Ng, S. P., Tsui, C. O., Roberts, D., Chau, P. Y., & Ng, M. H. (1996). Detection and serogroup differentiation of Salmonella spp. in food within 30 hours by enrichment-immunoassay with a T6 monoclonal antibody capture enzyme-linked immunosorbent assay. *Applied and environmental microbiology*, *62*(7), 2294-2302.

Nghiem, T. H. L., La, T. H., Vu, X. H., Chu, V. H., Nguyen, T. H., Le, Q. H., ... & Tran, H. N. (2010). Synthesis, capping and binding of colloidal gold nanoparticles to proteins. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, *1*(2), 025009.

Nie, S., & Emory, S. R. (1997). Probing single molecules and single nanoparticles by surface-enhanced Raman scattering. *science*, *275*(5303), 1102-1106.

Nieroda, C. A., Milenic, D. E., Carrasquillo, J. A., Schlom, J., & Greiner, J. W. (1995). Improved tumor radioimmunodetection using a single-chain Fv and γ-interferon: potential clinical applications for radioimmunoguided surgery and γ scanning. *Cancer research*, *55*(13), 2858-2865.

Nikaido, H. (2003). Molecular basis of bacterial outer membrane permeability revisited. *Microbiology and molecular biology reviews*, *67*(4), 593-656.

Norde, W. (1986). Adsorption of proteins from solution at the solid-liquid interface. *Advances in colloid and interface science*, *25*, 267-340.

Oberdörster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Kreyling, W., & Cox, C. (2004). Translocation of inhaled ultrafine particles to the brain. *Inhalation toxicology*, *16*(6-7), 437-445.

O'Farrell, B. (2009). Evolution in lateral flow–based immunoassay systems. In *Lateral Flow Immunoassay* (pp. 1-33). Humana Press.

O'Farrell B. (2013).Lateral Flow Immunoassay Systems: Evolution from the Current State of the Art to the Next Generation of Highly Sensitive, Quantitative Rapid Assays. Stratcom, Montreal, Canada. Personal Communication.

Oh, B. K., Lee, W., Chun, B. S., Bae, Y. M., Lee, W. H., & Choi, J. W. (2005). The fabrication of protein chip based on surface plasmon resonance for detection of pathogens. *Biosensors and Bioelectronics*, *20*(9), 1847-1850.

Oliver, C. (1999). Conjugation of colloidal gold to proteins. *Immunocytochemical Methods and Protocols*, 331-334.

Oliver, S. P., Jayarao, B. M., & Almeida, R. A. (2005). Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications.*Foodbourne Pathogens & Disease*, *2*(2), 115-129.

Olsen, S. J., Bishop, R., Brenner, F. W., Roels, T. H., Bean, N., Tauxe, R. V., & Slutsker, L. (2001). The changing epidemiology of Salmonella: trends in serotypes isolated from humans in the United States, 1987–1997. *Journal of Infectious Diseases*, *183*(5), 753-761.

Oral R., Sümer, S., Can, D., Tiraş, Ü., & Yener, H. (1995). Yenidoğan Ünitesinde Salmonella Typhimurium Salgını. *Turkiye Klinikleri Journal of Pediatrics*, *4*(2), 50-55.

O'Regan, E., McCabe, E., Burgess, C., McGuinness, S., Barry, T., Duffy, G., ... & Fanning, S. (2008). Development of a real-time multiplex PCR assay for the detection of multiple Salmonella serotypes in chicken samples. *BMC microbiology*, *8*(1), 1.

Padlan, E. A. (1990). On the nature of antibody combining sites: unusual structural features that may confer on these sites an enhanced capacity for binding ligands. *Proteins: Structure, Function, and Bioinformatics*, *7*(2), 112-124.

Paek, S. H., Lee, S. H., Cho, J. H., & Kim, Y. S. (2000). Development of rapid one-step immunochromatographic assay. *Methods*, *22*(1), 53-60.

Park, S., Worobo, R. W., & Durst, R. A. (2001). Escherichia coli O157: H7 as an emerging foodborne pathogen: a literature review. *Critical reviews in biotechnology*, *21*(1), 27-48.

Park, S. H., Jarquin, R., Hanning, I., Almeida, G., & Ricke, S. C. (2011). Detection of Salmonella spp. survival and virulence in poultry feed by targeting the hilA gene. *Journal of applied microbiology*, *111*(2), 426-432.

Pavlov, V., Xiao, Y., Shlyahovsky, B., & Willner, I. (2004). Aptamer-functionalized Au nanoparticles for the amplified optical detection of thrombin. *Journal of the American Chemical Society*, *126*(38), 11768-11769.

Peluso, P., Wilson, D. S., Do, D., Tran, H., Venkatasubbaiah, M., Quincy, D., ... & Witte, K. (2003). Optimizing antibody immobilization strategies for the construction of protein microarrays. *Analytical biochemistry*, *312*(2), 113-124.

Peng, H. I., & Miller, B. L. (2011). Recent advancements in optical DNA biosensors: exploiting the plasmonic effects of metal nanoparticles. *Analyst*, *136*(3), 436-447.

Pérez, F. G., Mascini, M., Tothill, I. E., & Turner, A. P. (1998). Immunomagnetic separation with mediated flow injection analysis amperometric detection of viable Escherichia coli O157. *Analytical chemistry*,*70*(11), 2380-2386.

Peruski, A. H., & Peruski, L. F. (2003). Immunological methods for detection and identification of infectious disease and biological warfare agents. *Clinical and diagnostic laboratory immunology*, *10*(4), 506-513.

Pillai, Z. S., & Kamat, P. V. (2004). What factors control the size and shape of silver nanoparticles in the citrate ion reduction method?. *The Journal of Physical Chemistry B*, *108*(3), 945-951.

Pleckaityte, M., Mistiniene, E., Lasickiene, R., Zvirblis, G., & Zvirbliene, A. (2011). Generation of recombinant single-chain antibodies neutralizing the cytolytic activity of vaginolysin, the main virulence factor of Gardnerella vaginalis. *BMC biotechnology*, *11*(1), 100.

Plückthun, A. (1991). Antibody Engineering: Advances from the use of Escherichia coli expression systems. *Biotechnology*, *9*, 545.

Plückthun, A., & Pack, P. (1997). New protein engineering approaches to multivalent and bispecific antibody fragments. *Immunotechnology*, *3*(2), 83-105.

Posthuma-Trumpie, G. A., Korf, J., & van Amerongen, A. (2009). Lateral flow (immuno) assay: its strengths, weaknesses, opportunities and threats. A literature survey. *Analytical and bioanalytical chemistry*, *393*(2), 569-582.

Prakash, S., Karacor, M. B., & Banerjee, S. (2009). Surface modification in microsystems and nanosystems. *Surface Science Reports*, *64*(7), 233-254.

Preechakasedkit, P., Pinwattana, K., Dungchai, W., Siangproh, W., Chaicumpa, W., Tongtawe, P., & Chailapakul, O. (2012). Development of a one-step immunochromatographic strip test using gold nanoparticles for the rapid detection of Salmonella typhi in human serum. *Biosensors and Bioelectronics*, *31*(1), 562-566.

Qu, Y., & Lü, X. (2009). Aqueous synthesis of gold nanoparticles and their cytotoxicity in human dermal fibroblasts–fetal. *Biomedical Materials*, *4*(2), 025007.

Radke, S. M., & Alocilja, E. C. (2005). A high density microelectrode array biosensor for detection of E. coli O157: H7. *Biosensors and Bioelectronics*, *20*(8), 1662-1667.

Raetz, C. R., & Whitfield, C. (2002). Lipopolysaccharide endotoxins. *Annual review of biochemistry*, *71*, 635.

Rahman, M. M., Guard-Petter, J., & Carlson, R. W. (1997). A virulent isolate of Salmonella enteritidis produces a Salmonella typhi-like lipopolysaccharide.*Journal of bacteriology*, *179*(7), 2126-2131.

Rahman, S., Lipert, R. J., & Porter, M. D. (2006). Rapid screening of pathogenic bacteria using solid phase concentration and diffuse reflectance spectroscopy. *Analytica chimica acta*, *569*(1), 83-90.

Rao, S. V., Anderson, K. W., & Bachas, L. G. (1998). Oriented immobilization of proteins. *Microchimica Acta*, *128*(3-4), 127-143.

Rao, V. K., Rai, G. P., Agarwal, G. S., & Suresh, S. (2005). Amperometric immunosensor for detection of antibodies of Salmonella typhi in patient serum. *Analytica chimica acta*, *531*(2), 173-177.

Rastogi, S. K., Gibson, C. M., Branen, J. R., Aston, D. E., Branen, A. L., & Hrdlicka, P. J. (2012). DNA Detection on Lateral Flow Test Strips: Enhanced Signal Sensitivity Using LNA-conjugated Gold Nanoparticles. *Chemical communications*, 48 (62), 7714-7716.

Rajtak, U., Leonard, N., Bolton, D., & Fanning, S. (2011). A real-time multiplex SYBR Green I polymerase chain reaction assay for rapid screening of Salmonella serotypes prevalent in the European Union. *Foodborne pathogens and disease*, *8*(7), 769-780.

Reiter, Y., & Pastan, I. (1998). Recombinant Fv immunotoxins and Fv fragments as novel agents for cancer therapy and diagnosis. *Trends in biotechnology*, *16*(12), 513-520.

Riley, L. W., Remis, R. S., Helgerson, S. D., McGee, H. B., Wells, J. G., Davis, B. R., ... & Blake, P. A. (1983). Hemorrhagic colitis associated with a rare Escherichia coli serotype. *New England Journal of Medicine*, *308*(12), 681-685.

Robinson, C. R., & Sauer, R. T. (1998). Optimizing the stability of single-chain proteins by linker length and composition mutagenesis. *Proceedings of the National Academy of Sciences*, *95*(11), 5929-5934.

Roffey, R., Lantorp, K., Tegnell, A., & Elgh, F. (2002). Biological weapons and bioterrorism preparedness: importance of public-health awareness and international cooperation. *Clinical microbiology and infection*, *8*(8), 522-528.

Ronholm, J., Zhang, Z., Cao, X., & Lin, M. (2011). Monoclonal antibodies to lipopolysaccharide antigens of Salmonella enterica serotype Typhimurium DT104. *Hybridoma*, *30*(1), 43-52.

Rosen, S. (2009). Market Trends in Lateral Flow Immunoassays Chapter 2. Lateral Flow Immunoassay. Humana Press, New York, (NY).

Rosi, N. L., Giljohann, D. A., Thaxton, C. S., Lytton-Jean, A. K., Han, M. S., & Mirkin, C. A. (2006). Oligonucleotide-modified gold nanoparticles for intracellular gene regulation. *Science*, *312*(5776), 1027-1030.

Rothenberg, E., Kazes, M., Shaviv, E., & Banin, U. (2005). Electric field induced switching of the fluorescence of single semiconductor quantum rods. *Nano letters*, *5*(8), 1581-1586.

Safenkova, I. V., Zherdev, A. V., & Dzantiev, B. B. (2010). Correlation between the composition of multivalent antibody conjugates with colloidal gold nanoparticles and their affinity. *Journal of immunological methods*, *357*(1), 17-25.

Saha, K., Agasti, S. S., Kim, C., Li, X., & Rotello, V. M. (2012). Gold nanoparticles in chemical and biological sensing. *Chemical reviews*, *112*(5), 2739-2779.

Salam, F. (2010). Development of immunosensors for salmonella typhimurium.

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). *Molecular cloning* (Vol. 2, pp. 14-9). New York: Cold spring harbor laboratory press.

Sapparapu, G. (2003). *Development of Immunological Reagents for Detecting Salmonella Enterica Serovar Typhimurium* (Master Of Science dissertation, University of Florida).

Scouten, W. H., Luong, J. H., & Brown, R. S. (1995). Enzyme or protein immobilization techniques for applications in biosensor design. *Trends in biotechnology*, *13*(5), 178-185.

Seo, K. H., Brackett, R. E., Hartman, N. F., & Campbell, D. P. (1999). Development of a rapid response biosensor for detection of Salmonella typhimurium. *Journal of Food Protection®*, *62*(5), 431-437.

Shaw, D. J. (1991). Colloid and Surface Chemistry. (Butterworth–Heinemann, Oxford).

Shaw, S. J., Blais, B. W., & Nundy, D. C. (1998). Performance of the Dynabeads anti-Salmonella system in the detection of Salmonella species in foods, animal feeds, and environmental samples. *Journal of Food Protection®*,*61*(11), 1507-1510.

Shen, G., Shen, G., & Yu, R. (2007). Au nanoparticle network-type thin films formed via mixed assembling and cross-linking route for biosensor application: Quartz crystal microbalance study. *Analytical biochemistry*, *365*(1), 1-6.

Sheridan, G. E. C., Masters, C. I., Shallcross, J. A., & Mackey, B. M. (1998). Detection of mRNA by Reverse Transcription-PCR as an Indicator of Viability in Escherichia coliCells. *Applied and Environmental Microbiology*, *64*(4), 1313-1318.

Shi, C., Zhao, S., Zhang, K., Hong ,G., Zhu, Z. (2008). Preparation of colloidal gold immunochromatography strip for detection of methamidophos residue. *J. Environ. Sci.*;20:1392–1397.

Shim, W. B., Song, J. E., Mun, H., Chung, D. H., & Kim, M. G. (2014). Rapid colorimetric detection of Salmonella typhimuriumusing a selective filtration technique combined with antibody–magnetic nanoparticle nanocomposites. *Analytical and bioanalytical chemistry*, *406*(3), 859-866.

Shiraishi, H., Itoh, T., Hayashi, H., Takagi, K., Sakane, M., Mori, T., & Wang, J. (2007). Electrochemical detection of E. coli 16S rDNA sequence using air-plasma-activated fullerene-impregnated screen printed electrodes. *Bioelectrochemistry*, *70*(2), 481-487.

Siegel, D. L. (2002). Recombinant monoclonal antibody technology. *Transfusion clinique et biologique*, *9*(1), 15-22.

Sigurskjold, B. W., Altman, E., & Bundle, D. R. (1991). Sensitive titration microcalorimetric study of the binding of Salmonella O-antigenic oligosaccharides by a monoclonal antibody. *European journal of biochemistry*, *197*(1), 239-246.

Sigurskjold, B. W., & Bundle, D. R. (1992). Thermodynamics of oligosaccharide binding to a monoclonal antibody specific for a Salmonella O-antigen point to hydrophobic interactions in the binding site. *Journal of Biological Chemistry*, *267*(12), 8371-8376.

Singh, C., Agarwal, G. S., Rai, G. P., Singh, L., & Rao, V. K. (2005). Specific detection of Salmonella typhi using renewable amperometric immunosensor. *electroanalysis*, *17*(22), 2062-2067.

Siragusa, G. R., & Johnson, M. G. (1990). Monoclonal antibody specific for Listeria monocytogenes, Listeria innocua, and Listeria welshimeri. *Applied and environmental microbiology*, *56*(6), 1897-1904.

Sithigorngul, W., Rukpratanporn, S., Sittidilokratna, N., Pecharaburanin, N., Longyant, S., Chaivisuthangkura, P., & Sithigorngul, P. (2007). A convenient immunochromatographic test strip for rapid diagnosis of yellow head virus infection in shrimp. *Journal of Virological Methods*, *140*(1), 193-199.

Skurnik, M., & Bengoechea, J. A. (2003). The biosynthesis and biological role of lipopolysaccharide O-antigens of pathogenic Yersiniae. *Carbohydrate research*, *338*(23), 2521-2529.

Slutsker, L., Altekruse, S. F., & Swerdlow, D. L. (1998). Foodborne DİseaseS: Emerging Pathogens and Trends** All material in this article, with the exception of borrowed figures, tables, or text, is in the public domain.*Infectious disease clinics of North America*, *12*(1), 199-216.

Sodoyer, R. (2004). Expression systems for the production of recombinant pharmaceuticals. *BioDrugs*, *18*(1), 51-62.

Song, C., Liu, Q., Zhi, A., Yang, J., Zhi, Y., Li, Q., ... & Zhang, G. (2011). Development of a lateral flow colloidal gold immunoassay strip for the rapid detection of olaquindox residues. *Journal of agricultural and food chemistry*, *59*(17), 9319-9326.

Song, C., Liu, C., Wu, S., Li, H., Guo, H., Yang, B., ... & Zhai, X. (2016). Development of a lateral flow colloidal gold immunoassay strip for the simultaneous detection of Shigella boydii and Escherichia coli O157: H7 in bread, milk and jelly samples. *Food Control*, *59*, 345-351.

Španová, A., Rittich, B., Karpíšková, R., Čechová, L., & Škapova, D. (2000). PCR identification of Salmonella cells in food and stool samples after immunomagnetic separation. *Bioseparation*, *9*(6), 379-384.

Sperling, R. A., Liedl, T., Duhr, S., Kudera, S., Zanella, M., Lin, C. A., ... & Parak, W. J. (2007). Size determination of (bio) conjugated water-soluble colloidal nanoparticles: a comparison of different techniques. *The Journal of Physical Chemistry C*, *111*(31), 11552-11559.

Sperling, R. A., Gil, P. R., Zhang, F., Zanella, M., & Parak, W. J. (2008). Biological applications of gold nanoparticles. *Chemical Society Reviews*, *37*(9), 1896-1908.

Stone, G. G., Oberst, R. D., Hays, M. P., McVey, S., Galland, J. C., Curtiss, R., ... & Chengappa, M. M. (1995). Detection of Salmonella typhimurium from rectal swabs of experimentally infected beagles by short cultivation and PCR-hybridization. *Journal of clinical microbiology*, *33*(5), 1292-1295.

Stuart, D. A., Haes, A. J., McFarland, A. D., Nie, S., & Van Duyne, R. P. (2004, June). Refractive-index-sensitive, plasmon-resonant-scattering, and surface-enhanced Raman-scattering nanoparticles and arrays as biological sensing platforms. Plasmonics in Biology and Medicine. 5327: 60-73.

Su, X. L., & Li, Y. (2004). A self-assembled monolayer-based piezoelectric immunosensor for rapid detection of Escherichia coli O157: H7. *Biosensors and Bioelectronics*, *19*(6), 563-574.

Suria, M. S., Mohd Afendy, A. T., Noor Azlina, M., & Zamri, I. (2015). Lateral flow assay strip for detection of Escherichia coli O157: H7. *International Food Research Journal*, *22*(6), 2587-2593.

Svenson, S. B., & Lindberg, A. A. (1978). Immunochemistry of Salmonella O-antigens: preparation of an octasaccharide-bovine serum albumin immunogen representative of Salmonella serogroup B O-antigen and characterization of the antibody response. *The Journal of Immunology*, *120*(5), 1750-1757.

Taban, B. M., Ben, U., & Aytac, S. A. (2009). Rapid detection of Salmonella in milk by combined immunomagnetic separation-polymerase chain reaction assay. *Journal of dairy science*, *92*(6), 2382-2388.

Tatumi, R., & Fujihara, H. (2005). Remarkably stable gold nanoparticles functionalized with a zwitterionic liquid based on imidazolium sulfonate in a high concentration of aqueous electrolyte and ionic liquid. *Chemical communications*, (1), 83-85.

Terpe, K. (2006). Overview of bacterial expression systems for heterologous protein production: from molecular and biochemical fundamentals to commercial systems. *Applied microbiology and biotechnology*, *72*(2), 211-222.

Thakur, M. S., & Ragavan, K. V. (2013). Biosensors in food processing. *Journal of food science and technology*, *50*(4), 625-641.

Thobhani, S., Attree, S., Boyd, R., Kumarswami, N., Noble, J., Szymanski, M., & Porter, R. A. (2010). Bioconjugation and characterisation of gold colloid-labelled proteins. *Journal of immunological methods*, *356*(1), 60-69.

Thompson, D. (2007). Michael Faraday's recognition of ruby gold: the birth of modern nanotechnology. *Gold Bulletin*, *40*(4), 267-269.

Tolner, B., Smith, L., Begent, R. H., & Chester, K. A. (2006a). Production of recombinant protein in Pichia pastoris by fermentation. *Nature protocols*, *1*(2), 1006-1021.

Tolner, B., Smith, L., Begent, R. H., & Chester, K. A. (2006b). Expanded-bed adsorption immobilized-metal affinity chromatography. *Nature protocols*, *1*(3), 1213-1222.

Tothill, I. E., & Turner, A. P. F. (2003). Biosensors. Encyclopaedia of food sciences and nutrition (second edition), 489-499, ISBN 0-12-227055-X.

Tripathi, V., Nara, S., Singh, K., Singh, H., & Shrivastav, T. G. (2012). A competitive immunochromatographic strip assay for 17-α-hydroxy progesterone using colloidal gold nanoparticles. *Clinica Chimica Acta*, *413*(1), 262-268.

Tsai, D. H., DelRio, F. W., Keene, A. M., Tyner, K. M., MacCuspie, R. I., Cho, T. J., ... & Hackley, V. A. (2011). Adsorption and conformation of serum albumin protein on gold nanoparticles investigated using dimensional measurements and in situ spectroscopic methods. *Langmuir*, *27*(6), 2464-2477.

Tsang, R. S., Chan, K. H., Lau, N. W., Choi, D. K., Law, D. K., & Ng, M. H. (1991). Characterization of murine monoclonal antibodies against serogroup B salmonellae and application as serotyping reagents. *Journal of clinical microbiology*, *29*(9), 1899-1903.

Turkevich, J., Stevenson, P. C., & Hillier, J. (1951). A study of the nucleation and growth processes in the synthesis of colloidal gold. *Discussions of the Faraday Society*, *11*, 55-75.

U.S. Food & Drug Administration. (2002). Escherichia coli O157:H7, in Foodborne Pathogenic Microorganisms and Natural Toxins Handbook. Center for Food Safety & Applied Nutrition.

Van Delden, R. A., Ter Wiel, M. K., Pollard, M. M., Vicario, J., Koumura, N., & Feringa, B. L. (2005). Unidirectional molecular motor on a gold surface. *Nature*, *437*(7063), 1337-1340.

Varshney, M., Li, Y., Srinivasan, B., & Tung, S. (2007). A label-free, microfluidics and interdigitated array microelectrode-based impedance biosensor in combination with nanoparticles immunoseparation for detection of Escherichia coli O157: H7 in food samples. *Sensors and Actuators B: Chemical*, *128*(1), 99-107.

Velusamy, V., Arshak, K., Korostynska, O., Oliwa, K., & Adley, C. (2010). An overview of foodborne pathogen detection: In the perspective of biosensors.*Biotechnology advances*, *28*(2), 232-254.

Verwey, E. J. W., Overbeek, J. T. G. (1948). The free energy of a double layer system. *Theory of the Stability of Lyophobic Colloids*. Elsevier, New York,p. 51.

Voetsch, A. C., Van Gilder, T. J., Angulo, F. J., Farley, M. M., Shallow, S., Marcus, R., & Tauxe, R. V. (2004). Emerging Infections Program FoodNet Working Group. FoodNet estimate of the burden of illness caused by nontyphoidal Salmonella infections in the United States. *Clin Infect Dis*,*38*(Suppl 3), 127-34.

Waldmann, T. A. (1991). Monoclonal antibodies in diagnosis and therapy.*Science*, *252*(5013), 1657-1662.

Wallace, D. J., Van Gilder, T., Shallow, S., Fiorentino, T., Segler, S. D., Smith, K. E., ... & Angulo, F. J. (2000). Incidence of foodborne illnesses reported by the foodborne diseases active surveillance network (FoodNet)—1997. *Journal of Food Protection®*, *63*(6), 807-809.

Wang, J. (1998). DNA biosensors based on peptide nucleic acid (PNA) recognition layers. A review. *Biosensors and Bioelectronics*, *13*(7), 757-762.

Wang, J. (1999). PNA biosensors for nucleic acid detection. *Curr Issues Mol Biol*, *1*, 117-122.

Wang, J., Ensor, C. M., Dubuc, G. J., Narang, S. A., & Daunert, S. (2001). Genetically fused single-chain anti-Salmonella antibody with aequorin: a bioluminescence immunoassay for a Salmonella antigen. *Analytica chimica acta*, *435*(2), 255-263.

Wang, S. H., Zhang, J. B., Zhang, Z. P., Zhou, Y. F., Yang, R. F., Chen, J., ... & Zhang, X. E. (2006). Construction of Single Chain Variable Fragment (ScFv) and BiscFv-Alkaline Phosphatase Fusion Protein for Detection of Bacillus A nthracis. Analytical chemistry, 78(4), 997-1004.

Wang, L., Li, Y., & Mustapha, A. (2007). Rapid and simultaneous quantitation of Escherichia coli O157: H7, Salmonella, and Shigella in ground beef by multiplex real-time PCR and immunomagnetic separation. *Journal of Food Protection®*, *70*(6), 1366-1372.

Wang, Y., Ye, Z., Si, C., & Ying, Y. (2011a). Subtractive inhibition assay for the detection of E. coli O157: H7 using surface plasmon resonance. *Sensors*,*11*(3), 2728-2739.

Wang, R. E., Zhang, Y., Cai, J., Cai, W., & Gao, T. (2011b). Aptamer-based fluorescent biosensors. *Current medicinal chemistry*, *18*(27), 4175.

Wang, Y., Zhang, X., Zhang, C., Liu, Y., & Liu, X. (2012). Isolation of single chain variable fragment (scFv) specific for Cry1C toxin from human single fold scFv libraries. *Toxicon*, *60*(7), 1290-1297.

Wang, C., Zhang, L., & Shen, X. (2013). Development of a nucleic acid lateral flow strip for detection of hepatitis c virus (Hcv) core antigen. *Nucleosides, Nucleotides and Nucleic Acids*, *32*(2), 59-68.

Wannlund, J., Azari, J., Levine, L., & DeLuca, M. (1980). A bioluminescent immunoassay for methotrexate at the subpicomole level. *Biochemical and biophysical research communications*, *96*(1), 440-446.

Waswa, J., Irudayaraj, J., & DebRoy, C. (2007). Direct detection of E. coli O157: H7 in selected food systems by a surface plasmon resonance biosensor. *LWT-Food Science and Technology*, *40*(2), 187-192.

Wells, S., Fedorka-Cray, P. J., Besser, T., McDonough, P., & Smith, B. (1998). E. coli O157: H7 and Salmonella–status on US dairy operations. *Centers for Epidemiology and Animal Health. Fort Collins, CO: USDA/APHIS/VS*.

Wels, W., Harwerth, I. M., Zwickl, M., Hardman, N., Groner, B., & Hynes, N. E. (1992). Construction, Bacterial Expression and Characterization of a Bifunctional Single–Chain Antibody–Phosphatase Fusion Protein Targeted to the Human ERBB–2 Receptor. *Nature Biotechnology*, *10*(10), 1128-1132.

Wemmer, S., Mashau, C., Fehrsen, J., Van Wyngaardt, W., & Du Plessis, D. H. (2010). Chicken scFvs and bivalent scFv-C H fusions directed against HSP65 of Mycobacterium bovis. *Biologicals*, *38*(3), 407-414.

Wen, C. Y., Hu, J., Zhang, Z. L., Tian, Z. Q., Ou, G. P., Liao, Y. L., ... & Pang, D. W. (2013). One-step sensitive detection of Salmonella typhimurium by coupling magnetic capture and fluorescence identification with functional nanospheres. *Analytical chemistry*, *85*(2), 1223-1230.

West, J. L., & Halas, N. J. (2003). Engineered nanomaterials for biophotonics applications: improving sensing, imaging, and therapeutics. *Annual Review of Biomedical Engineering*, *5*(1), 285-292.

Wilkinson, S. G. (1977). Composition and structure of bacterial lipopolysaccharides. In *Surface carbohydrates of the prokaryotic cell* (pp. 97-175). Academic Press New York.

Wilson, O. M., Hu, X., Cahill, D. G., & Braun, P. V. (2002). Colloidal metal particles as probes of nanoscale thermal transport in fluids. *Physical Review B*, *66*(22), 224301.

Woods, D. F., Reen, F. J., Gilroy, D., Buckley, J., Frye, J. G., & Boyd, E. F. (2008). Rapid multiplex PCR and real-time TaqMan PCR assays for detection of Salmonella enterica and the highly virulent serovars Choleraesuis and Paratyphi C. *Journal of clinical microbiology*, *46*(12), 4018-4022.

Wu, V. C., Chen, S. H., & Lin, C. S. (2007). Real-time detection of Escherichia coli O157: H7 sequences using a circulating-flow system of quartz crystal microbalance. *Biosensors and Bioelectronics*, *22*(12), 2967-2975.

Wu, W., Li, J., Pan, D., Li, J., Song, S., Rong, M., ... & Lu, J. (2014). Gold nanoparticle-based enzyme-linked antibody-aptamer sandwich assay for detection of Salmonella Typhimurium. *ACS applied materials & interfaces*, *6*(19), 16974-16981.

Wulandari, P., Li, X., Tamada, K., & Hara, M. (2008). Conformational study of citrates adsorbed on gold nanoparticles using Fourier transform infrared spectroscopy. *Journal of Nonlinear Optical Physics & Materials*, *17*(02), 185-192.

Xia, S., Yu, Z., Liu, D., Xu, C., & Lai, W. (2016). Developing a novel immunochromatographic test strip with gold magnetic bifunctional nanobeads (GMBN) for efficient detection of Salmonella choleraesuis in milk. *Food Control*, *59*, 507-512.

Xu, Q., Xu, H., Gu, H., Li, J., Wang, Y., & Wei, M. (2009). Development of lateral flow immunoassay system based on superparamagnetic nanobeads as labels for rapid quantitative detection of cardiac troponin I. *Materials Science and Engineering: C*, *29*(3), 702-707.

Yang, L., Li, Y., & Erf, G. F. (2004). Interdigitated Array Microelectrode-Based Electrochemical Impedance Immunosensor for Detection of Escherichia c oli O157: H7. *Analytical Chemistry*, *76*(4), 1107-1113.

Yang, L., & Li, Y. (2006). Simultaneous detection of Escherichia coli O157：H7 and Salmonella Typhimurium using quantum dots as fluorescence labels.*Analyst*, *131*(3), 394-401.

Yang, L., Mao, H., Wang, Y. A., Cao, Z., Peng, X., Wang, X., ... & Smith, M. Q. (2009). Single chain epidermal growth factor receptor antibody conjugated nanoparticles for in vivo tumor targeting and imaging. Small, 5(2), 235-243.

Yang, Y., Xu, F., Xu, H., Aguilar, Z. P., Niu, R., Yuan, Y., ... & Wan, C. (2013). Magnetic nano-beads based separation combined with propidium monoazide treatment and multiplex PCR assay for simultaneous detection of viable Salmonella Typhimurium, Escherichia coli O157: H7 and Listeria monocytogenes in food products. *Food microbiology*, *34*(2), 418-424.

Yanik, A. A., Huang, M., Artar, A., Chang, T. Y., & Altug, H. (2010). Integrated nanoplasmonic-nanofluidic biosensors with targeted delivery of analytes. *Applied physics letters*, *96*(2), 021101.

Yao, J. L., Pan, G. P., Xue, K. H., Wu, D. Y., Ren, B., Sun, D. M., ... & Tian, Z. Q. (2000). A complementary study of surface-enhanced Raman scattering and metal nanorod arrays. *Pure and applied chemistry*, *72*(1-2), 221-228.

Yaron, S., & Matthews, K. R. (2002). A reverse transcriptase-polymerase chain reaction assay for detection of viable Escherichia coli O157: H7: investigation of specific target genes. *Journal of applied microbiology*, *92*(4), 633-640.

Ye, R. (2010). Nanoparticle-amplified immunobiosensor enables excellent sensitivity in rapid detection of viable E. coli O157: H7. *Stockholm Junior Water Prize*.

Yin, J., Li, G., Ren, X., & Herrler, G. (2007). Select what you need: a comparative evaluation of the advantages and limitations of frequently used expression systems for foreign genes. *Journal of Biotechnology*, *127*(3), 335-347.

Yokota, T., Milenic, D. E., Whitlow, M., & Schlom, J. (1992). Rapid tumor penetration of a single-chain Fv and comparison with other immunoglobulin forms. *Cancer research*, *52*(12), 3402-3408.

Young, N. M., Watson, D. C., Cunningham, A. M., & MacKenzie, C. R. (2014). The intrinsic cysteine and histidine residues of the anti-Salmonella antibody Se155-4: a model for the introduction of new functions into antibody-binding sites. *Protein Engineering Design and Selection*, 27(10), 383-390.

Yu, L., Wilfred, C. & Ashok, M. (2006). Microbial biosensors, *Analytica Chimica Acta*, 568 (1-2), 200-210.

Yu, C. Y., Ang, G. Y., Chua, A. L., Tan, E. H., Lee, S. Y., Falero-Diaz, G., ... & Sarmiento, M. E. (2011). Dry-reagent gold nanoparticle-based lateral flow biosensor for the simultaneous detection of Vibrio cholerae serogroups O1 and O139. *Journal of microbiological methods*, *86*(3), 277-282.

Yuan, J., Tao, Z., Yu, Y., Ma, X., Xia, Y., Wang, L., & Wang, Z. (2014). A visual detection method for Salmonella typhimurium based on aptamer recognition and nanogold labeling. *Food Control*, *37*, 188-192.

Zaytseva, N. V., Goral, V. N., Montagna, R. A., & Baeumner, A. J. (2005). Development of a microfluidic biosensor module for pathogen detection. *Lab on a Chip*, *5*(8), 805-811.

Zdanov, A., Li, Y., Bundle, D. R., Deng, S. J., MacKenzie, C. R., Narang, S. A., ... & Cygler, M. (1994). Structure of a single-chain antibody variable domain (Fv) fragment complexed with a carbohydrate antigen at 1.7-A resolution. *Proceedings of the National Academy of Sciences*, *91*(14), 6423-6427.

Zeng, Q., Mao, X., Xu, H., Wang, S., & Liu, G. (2009). Quantitative immunochromatographic strip biosensor for the detection of carcinoembryonic antigen tumor biomarker in human plasma. *Am. J. Biomed. Sci*, *1*(1), 70-79.

Zhang, P., & Bundle, D. R. (2000). Synthesis of three tethered trisaccharides to probe entropy contributions in carbohydrate—protein interactions. *Israel Journal of Chemistry*, *40*(3-4), 189-208.

Zhang, S., Huang, T. S., Bridgman, R., & Weese, J. (2006a). Development and characterization of monoclonal and polyclonal antibodies against Salmonella enterica Typhimurium for biosensor detection. *Journal of food science*, *71*(3), M100-M104.

Zhang, G. P., Wang, X. N., Yang, J. F., Yang, Y. Y., Xing, G. X., Li, Q. M., ... & Guo, J. Q. (2006b). Development of an immunochromatographic lateral flow test strip for detection of β-adrenergic agonist clenbuterol residues. *Journal of immunological methods*, *312*(1), 27-33.

Zhang, D., & Alocilja, E. C. (2008). Characterization of nanoporous silicon-based DNA biosensor for the detection of Salmonella Enteritidis. *Sensors Journal, IEEE*, *8*(6), 775-780.

Zhang, G., Guo, J., & Wang, X. (2009). Immunochromatographic lateral flow strip tests. *Biosensors and Biodetection: Methods and Protocols: Electrochemical and Mechanical Detectors, Lateral Flow and Ligands for Biosensors*, 169-183.

Zhang, D., Li, P., Zhang, Q., & Zhang, W. (2011). Ultrasensitive nanogold probe-based immunochromatographic assay for simultaneous detection of total aflatoxins in peanuts. *Biosensors and Bioelectronics*, *26*(6), 2877-2882.

Zhang, D. H., Li, P. W., Zhang, Q., Yang, Y., Zhang, W., Guan, D., & Ding, X. X. (2012). Extract-free immunochromatographic assay for on-site tests of aflatoxin M1 in milk. *Analytical Methods*, *4*(10), 3307-3313.

Zhang, L., Huang, Y., Wang, J., Rong, Y., Lai, W., Zhang, J., & Chen, T. (2015). Hierarchical flowerlike gold nanoparticles labeled immunochromatography test strip for highly sensitive detection of Escherichia coli O157: H7. Langmuir, 31(19), 5537-5544.

Zhao, Y., Zhang, G., Liu, Q., Teng, M., Yang, J., & Wang, J. (2008). Development of a lateral flow colloidal gold immunoassay strip for the rapid detection of enrofloxacin residues. *Journal of agricultural and food chemistry*, *56*(24), 12138-12142.

Zhao, Y., Ye, M., Chao, Q., Jia, N., Ge, Yu., & Shen, H. (2009). Simultaneous detection of multifood-borne pathogenic bacteria based on functionalized quantum dots coupled with immunomagnetic separation in food samples. Journal of Agricultural and Food Chemistry, 57(2), 517-524.

Zhao, X., He, X., Li, W., Liu, Y., Yang, L., & Wang, J. (2010). Development and evaluation of colloidal gold immunochromatographic strip for detection of Escherichia coli O157. *African Journal of Microbiology Research*, *4*(9), 663-670.

Zhu, H., Sikora, U., & Ozcan, A. (2012). Quantum dot enabled detection of Escherichia coli using a cell-phone. *Analyst*, *137*(11), 2541-2544.

Zuo, P., Li, X., Dominguez, D. C., & Ye, B. C. (2013). A PDMS/paper/glass hybrid microfluidic biochip integrated with aptamer-functionalized graphene oxide nano-biosensors for one-step multiplexed pathogen detection. *Lab on a Chip*, *13*(19), 3921-3928.

CURRICULUM VITAE

PERSONAL INFORMATION

Surname, Name **:** ÇAM Dilek

Birth : Februrary 22, 1983, Kahramanmaraş, Turkey

Nationality : Republic of Turkey (TC)

Address : METU Dept. of Biological Sciences
ODTÜ Biyolojik Bilimler Bölümü
Ankara, 06800 Turkey

Phone : +90 312 210 5156

Mobile : +90 533 554 0742

e-mail **:** dicam@metu.edu.tr
cam.dilek@gmail.com

EDUCATION

2010 - 2016 PhD - METU, Dept. of Biological Sciences

2009 - 2010 English Proficiency - METU, School of Foreign Languages

2007 - 2009 MSc - Gazi University, Dept. of Biology

2002 - 2007 BS - Gazi University, Dept. of Biological Education

RESEARCH INTEREST

Nanobiotechnology, Nanotoxicity, nanobiosensors

M.SC. THESIS

Cellular Uptake And Cytotoxicity Of Modified Silver Nanoparticles

WORK EXPERIENCES

2010 - 2016 Research Assistant, METU, Faculty Development Program (ÖYP)

2010 - 2016 Teaching Assistant, METU, Dept. of Biological Sciences

2014 - 2015 Visiting scholar at the University of Texas at Austin

2013 - 2014 Voluntary educator to the students of İlkyar schools, METU, Dep. of Biology

2008 - 2009 Research and Teaching Assistant, University of K.Maraş Sütçü İmam, Dep. of Biology

2008 – 2009 Researcher at Yeditepe University, Nanotechnology Research Group

2007 - 2008 Teaching Assistant, Gazi University, Dep. of Biology Education

PUBLICATIONS

1. Erdinç SEZGİN, Ömer Faruk KARATAŞ, **Dilek ÇAM**, İlknur SUR, İsmail SAYIN, Ertuğ AVCI, Kemal KESEROĞLU, Selim SÜLEK, Mustafa ÇULHA. 2008. Interaction of Gold Nanoparticles With Living Cells. Sigma Vol./26 Issue 4

2. **Dilek Cam**, Kemal Keseroglu, Mehmet Kahraman, Fikrettin Sahin and Mustafa Culha. Multiplex identification of bacteria in bacterial mixtures with surface-enhanced Raman scattering. J. Raman Spectrosc. 2010, 41, 484–489

3. Ilknur Sur, **Dilek Cam**, Mehmet Kahraman, Aslı Baysal and Mustafa Culha. Interaction of multi-functional silver nanoparticles with living cells. Nanotechnology 21, (2010), 175104 (10pp)

4. Mustafa Culha, Mehmet Kahraman, **Dilek Cam**, Ismail Sayın and Kemal Keseroglu. Rapid identification of bacteria and yeast using surface-enhanced Raman scattering. Surf. Interface Anal. 2010, 42, 462–465

5. Small molecule detection by lateral flow strips via aptamer-gated silica nanoprobes, V. Cengiz Özalp, **Dilek Çam**, Frank J Hernandez, Luiza I Hernandez, Thomas Schafer, Huseyin A. Oktem. DOI: 10.1039/C6AN00273K. Analyst, 2016, 141, 2595-2599

PRESENTATIONS IN AN INTERNATIONAL CONFERENCES

1. Cigdem Alev Ozel, İlknur Sur, M.Alev Ateş, Funda Ozdemir, **Dilek Cam**, Khalid Mahmood Khawar, Orhan Arslan. "Efficient in vitro micropropagation of Ornithogalum oligophyllum E.D clarke", Botanical Congress, University of Hamburg, Germany September 3-7, 2007 (Poster presentation)

2. Khalid Mahmood Khawar, Cigdem Alev Ozel, Secil Yildrim, Funda Ozdemir, **Dilek Cam**, Fatos Uzuntas, Orhan Arslan and Sebahattin Ozcan. "Factors affecting in vitro plant regeneration of Iranian knapweed (Centaurea depressa Bieb.) using immature zygotic embryos". Fifth International Scientific Conference "Propagation of Ornamental Plants", September, 5-8, 2007 Sofia - Bulgaria (Oral presentation)

3. Khawar KM, Ozel CA, Ulug A, Sur I, Kizilates E, Hadimogulları B, Ozlu G, **Cam D**, Arslan O., "Rapid and Highly efficient micropropagation of Two Turkish woads Isatis constricta Davis and I. aucheri Boiss. under in vitro conditions" International Symposium on Biotechnology, Sfax, Tunus, May 4-8 2008 (Oral presentation).

4. Mustafa Culha, Mehmet Kahraman, **Dilek Cam**, Dilek Gelen, Merve Yüksel, Rawil Fakhrullin, Fikrettin Sahin. "Multiplex Identification of Bacteria Using Surface Enhanced Raman Scattering". Pittcon 2009 (Oral Presentation)

5. Mustafa Çulha, Rawil F. Fakhrullin, Alsu I. Zamaleeva, Mehmet Kahraman, Dilek Gelen, **Dilek Çam**, Fikrettin Şahin, Merve Yüksel, "Engineering of gold and Silver nanoparticles into bacterial cell wall". Nanotech Europe, Berlin, Germany, September 28-30, 2009. (Oral presentation)

6. M. Culha, M. Kahraman, **D. Çam**, İ. Sayın, K. Keseroğlu. "Fast Identification of Bacteria and Yeast Using Surface-Enhanced Raman Scattering". 13th European Conference on Applications of Surface and Interface Analysis" Antalya, Turkey October 18-23, 2009 (Oral presentation)

7. **Dilek CAM**, Hüseyin Avni OKTEM, "Development of Gold Nanoparticle Based Lateral Flow Test Platform for Rapid Detection of Food Pathogens". 3rd International Conference and Exhibition on Biosensors & Bioelectronics, San Antonio/USA, August 11-13, 2014 (Poster presentation)

PRESENTATIONS IN A NATIONAL CONFERENCES

1. Özel Çiğdem Alev, Khawar KM, Keskin Nilay, Ozdemir Funda, Ates Mevlüde Alev, **Cam Dilek**, Arslan Orhan, Özcan Sebahattin, Ünal Fatma. "In vitro generation of Muscari macrocarpum". XVIII. National Biology Congress - Adnan Menderes University Faculty of Science and Art Department of Biological Sciences, Kuşadası/Aydın, 26-30 June 2006 (Poster presentation)

2. **Dilek Cam**, Funda Ozdemir, Çiğdem Alev Özel, Khalid Mahmood Khawar, Orhan Arslan Khawar. "Somatic embriyogenesis of wild lentil (Lens Culinaris Orientalis)". XIII. National Biology Student Congress, Ege University, İzmir 20-23 September 2006, s.18 (Oral presentation)

3. **Dilek Çam**, Ahmet Doğan, Orhan Arslan, Khalid Mahmood Khawar, Çiğdem Alev Özel. "In vitro micropropagation of endemic Muscari massayanum". XIV. National Biology Student Congress, Selçuk University / Konya, 3-4-5 September 2007 (Oral presentation)

4. Khalid Mahmood Khawar, Çiğdem Alev Özel, Mevlüde Alev Ateş, **Dilek Çam**, Funda Özdemir. "In vitro behaviour putting out roots of Ornithogalum ulophyllum Hand-Mazz" , XV. Biotechnology Congress, Akdeniz University/Antalya, 28-31 October 2007 (Poster presentation)

5. Ilknur Sur, Didem Sevim Mengu, Reinhard Lieberei, **Dilek Çam**, Orhan Arslan, Khalid Mahmood Khawar., "Temporary immersion system for micropropagation of Gentiana scabra", 4. National Nanoscience ve Nanotechnology Conference (NanoTr4), İstanbul Technical University, İstanbul , 9-13 June 2008 (Poster presentation)

6. Funda Özdemir, **Dilek Çam**, M. Alev Ateş, Çiğdem Alev Özel, Khalid Mahmood Khawar."Propagation of Urginea maritima (L.) BAKER (ada soğanı) with way of plant tissue culture". XIX. National Biology Congress. Karadeniz Technical University. Osman Turan Congress and Culture Center, Trabzon 23 - 27 June 2008 (Poster presentation)

7. İlknur Sur, **Dilek Çam**, Mehmet Kahraman, Mustafa Çulha. "Differential Targeting Cancer Cells with Modified Silver Nanoparticles". 5th Nanoscience and Nanotechnology Conference, June 08-12, 2009, Eskişehir/Türkiye (Oral Presentation)

8. **Dilek Cam**, Kemal Keseroglu, Mehmet Kahraman, Fikrettin Sahin, Mustafa Culha. "Identification of Bacterial Mixtures Based on SERS". 5th Nanoscience and Nanotechnology Conference, June 08-12, 2009, Eskişehir/Türkiye (Poster presentation)

9. **Dilek ÇAM**, Hüseyin Avni ÖKTEM. "A Novel DNA Sandwich Hybridization Based Detection Platform for Telomerase Activity". NanoTR-9, 24-28 June 2013 Erzurum / Türkiye (Poster presentation)

10. **Dilek ÇAM**, Hüseyin Avni ÖKTEM. "Rapidly and sensitively detection of pathogenic *E.coli* O157:H7 by developed lateral flow strip based on gold nanoparticles". NanoTR-11 (XI. Nanoscience and Nanotechnology Congress). Ankara/Türkiye, June 22-25, 2015 (Oral presentation)

11. **Dilek ÇAM**, Hüseyin Avni ÖKTEM. "Development of Lateral Flow Test Platform for rapid and sensitive detection of food pathogens". XVIII. National Biotechnology Congress. Konya/Türkiye, December 18-19, 2015 (Oral presentation)

COURSES&CONGRESS&SYMPOSIUMS

1. Food Security, Research of the importance of food security on account of biological diversity and determination of the suggestions to the Biosecurity, Ankara/Türkiye, October 20-22, 2005.

2. The course of DNA Markers and their use in plant breeding, Çukurova University-Adana/Türkiye, January 19–20, 2006.

3. Biology Display for the 80.year activity of Gazi University, Ankara/Türkiye, May 8–9–10 2006, (Attendant/Participant)

4. XVIII. National Biology Congress, Kuşadası/Aydın/Türkiye, June 26-30, 2006

5. VII. National Natural Sciences and Math Education congress, Ankara/Türkiye, September 7-9, 2006, (Attendant/Participant)

6. XIII. National Biology Student Congress, Ege University, İzmir/Türkiye, September 20-23, 2006

7. IV. National Molecular Biology and Genetics Winter School for Students, İstanbul University, İstanbul/Türkiye, February 1-4, 2007.

8. 3rd Traditional Natural Sciences and Maths Display at Gazi University, Ankara/Türkiye, May 8-9-10, 2007, (Attendant/Participant)

9. XV. Biotechnology Congress, Antalya/Türkiye, October 28-31, 2007

10. IV. Nanosicence and Nanotechnology Conference (Nano TR IV), İstanbul/Türkiye, June, 9-13, 2008

11. XIX. National Biology Congress, Trabzon/Türkiye, June 23 – 27, 2008

12. Joint Oncology Symposium, İstanbul/Türkiye, March 26-27, 2009

13. Nanomats: International Conference on Nanomaterials and Nanosystems, İTÜ, İstanbul/Türkiye, August 10-13, 2009

14. 6th Nanoscience and Nanotechnology Conference, Çeşme/İzmir/Türkiye, 15-18 June 2010

15. Potsdam Days on Bioanalysis 2011. Germany, November 9-10

16. VIII. Nanoscience and Nanotechnology Congress (NANO-TR8), Ankara/Türkiye, June 25-29, 2012

17. 15th European Congress on Biotechnology (bio-crossroads), İstanbul/Türkiye, 23-26 September 2012

18. Biological Threat Detection and Identification Technologies: Health, Environment and CBRN Applications December 11, 2012 METU/Ankara/Türkiye

19. Antibody days (Ankara Biotechnology Days), Ankara/Türkiye, 20 May 2015

MEMBERSHIPS

Member of Executive Board of Turkish Biotechnology Association.

SCHOLARSHIPS

2014 - 2015	Research scholarship for gradute students of Scientific and Technological Research Council of Turkey at abroad (TÜBİTAK-2214A)
2010 - 2015	Scholarship for gradute students of Scientific and Technological Research Council of Turkey (TÜBİTAK - 2211)
2006 - 2007	Scholarship of Prime ministry of Turkey
2006 - 2007	Achievement grant of Gazi University

AWARDS

1. The best poster presentation : "Development of Gold Nanoparticle Based Lateral Flow Test Platform for Rapid Detection of Food Pathogens". 3rd International Conference and Exhibition on Biosensors & Bioelectronics, San Antonio/USA, August 11-13, 2014

2. Encouragement award supplied by TÜBİTAK for publication (J. Raman Spectrosc. 2010, 41, 484–489)

3. Encouragement award supplied by TÜBİTAK for publication (Nanotechnology 21, (2010), 175104 (10pp))

4. Encouragement award supplied by TÜBİTAK for publication (Surf. Interface Anal. 2010, 42, 462–465)