MARMARA ÜNİVERSİTESİ
FEN BİLİMLERİ ENSTİTÜSÜ

SU KAYNAKLARININ MANYETİK NANO PARÇACIKLARLA ESCHERICHIA COLI' DEN ARINDIRILMASI

MERVE PINAR TOPBAŞ TEKİN

YÜKSEK LİSANS TEZİ
Fizik Anabilim Dalı
Fizik Programı

DANIŞMAN
Doç. Dr. Fatih DUMLUDAĞ

EŞ-DANIŞMAN
Doç. Dr. Çiğdem ORUÇ

İSTANBUL, 2019

MARMARA UNIVERSITY
INSTITUTE FOR GRADUATE STUDIES
IN PURE AND APPLIED SCIENCES

REDUCTION OF ESCHERICHIA COLI POPULATIONS IN WATER SAMPLES BY USING MAGNETIC NANOPARTICLES

MERVE PINAR TOPBAŞ TEKİN
(521214007)

MASTER THESIS
Department of Physics

Thesis Supervisor
Assoc. Prof. Dr. Fatih DUMLUDAĞ

Thesis CO- Supervisor
Assoc. Prof. Dr. Çiğdem ORUÇ

ISTANBUL, 2019

MARMARA UNIVERSITY
INSTITUTE FOR GRADUATE STUDIES IN PURE AND APPLIED SCIENCES

Merve Pınar TOPBAŞ TEKİN, a Master of Science student of Marmara University Institute for Graduate Studies in Pure and Applied Sciences, defended her thesis entitled "REDUCTION OF ESCHERICHIA COLI POPULATIONS IN WATER SAMPLES BY USING MAGNETIC NANOPARTICLES", on .....2019 and has been found to be satisfactory by the jury members.

Jury Members

Assoc. Prof. Dr. Fatih DUMLUDAĞ (Advisor)
Marmara University .....(SIGN) .....

Prof. Dr. Zikri ALTUN(Jury Member)
Marmara University .....(SIGN) .....

Prof. Dr. Ahmet ALTINDAL(Jury Member)
Yıldız Technical University .....(SIGN) .....

APPROVAL

Marmara University Institute for Graduate Studies in Pure and Applied Sciences Executive Committee approves that Merve Pınar Topbaş Tekin is granted the degree of Master of Science of Physics in Department of Physics, Physics Program on 10 July 2019. (Resolution no: 2019/114-02).

Director of the Institute
Prof. Dr.Bülent EKİCİ

TEŞEKKÜR

Bu tezin yazımında beni yönlendiren ve desteğini esirgemeyen değerli tez danışmanım Doç. Dr. Fatih Dumludağ ve Doç. Dr. Çiğdem Oruç'a sonsuz teşekkür ederim. Yıldız Teknik Üniversitesi organik teknolojiler labaratuarı bünyesinde bu çalışmayı yapmama olanak sağlayan Yıldız Teknik Üniversitesi Fizik bölüm başkanı Prof. Dr. Ahmet Altındal'a çok teşekkür ederim. Beni yetiştirip büyüterek bu seviyeye gelmemi sağlayan, sevgisini ve desteğini esirgemeyen annem Canan Topbaş'a, babam Mehmet Topbaş'a ve karamsarlığa düştüğümde beni cesaretlendirip motive eden sevgili eşim Özgür İsa Tekin'e teşekkürü bir borç bilirim.

Haziran, 2019 **Merve Pınar TOPBAŞ TEKİN**

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ÖZET

Su Kaynaklarının Manyetik Nano Parçacıklarla Escherichia Coli' den Arındırılması

Tek hücreli mikro-organizmalar olan bakteriler dünyadaki biyokütlenin en ağırlıklı kısmını oluştururlar ve yaklaşık olarak bir gram toprakta 40 milyon, 1 mililitre tatlı suda yaklaşık 1 milyon bakteri hücresi bulunur. Bakterilerin bazıları bağışıklık sistemimizi koruyucu probiyotik etkiye sahip olsalar da çoğu bakterilerin hastalık yapıcı etkiye sahip oldukları da iyi bilinmektedir. Bugün, patojenik bakteriler, virüsler ve parazitler gibi besin ve içme suyu yoluyla alınan mikro-organizmaların neden olduğu 250'den fazla hastalık olduğu bilinmektedir. Bu hastalık yapıcı mikro-organizmalar arasında bakteriler %91'lik bir oranla en büyük paya sahiptirler. Dünya'daki kullanılabilir su kaynaklarının sınırlı olmasından dolayı, su kaynaklarının etkin kullanımını sağlamak için su kaynaklarının özellikle *Escherichia coli* (*E. coli*) gibi bakterilerden arındırılması büyük önem arz etmektedir.

Bu çalışmada, özellikle su kaynaklarındaki *E. coli* bakteri sayısının azaltılmasına yönelik manyetik özellik gösteren (dışarıdan uygulanan manyetik alan ile kontrol edilebilen) nano parçacıkların kullanılabilirliğini göstermek amacıyla bir yöntem önerilmiştir. Önerilen yöntem temelde kısaca, manyetik özellik gösteren demir oksit nano parçacıkların sentezini, üretilen nano parçacıkların yüzeyinin biotin, streptavidin, biotin+ streptavidin, biotin+streptavidin+aptamer ile fonksiyonel hale getirilmesini, bu yapılara E.coli bakterilerinin bağlanmasını sağlayarak, su numunelerinin *E. coli* bakterilerinden arındırılmasını ve söz konusu modifiye manyetik nano parçacıklarına *E. coli* bakterilerinin bağlama miktarının Flowsitometri yardımı ile incelenmesini kapsamaktadır.

Konu ile ilgili yapılan gerek ön gerekse ayrıntılı çalışmalar modifiye ve/veya modifiye olmayan manyetik nano parçacıkların su kaynaklarının *E. coli* bakterilerinden arındırılması konusunda oldukça büyük bir potansiyele sahip olduklarını göstermektedir.

Anahtar Kelimeler: Escherichia coli, demir oksit , nano parçacık, streptavidin, biotin, aptamer.

ABSTRACT

Reduction of Escherichia coli Populations in Water Samples by Using Magnetic Nanoparticles

Bacteria, which are single-celled micro-organisms, constitute the greater part of the biomass in the world and may contain 40 million bacteria cell in 1 g soil and 1 million bacteria cell in 1 ml fresh water. Although some of the bacteria have a protective probiotic effect on our immune system, it is also well known that most of the bacteria have disease-causing effects. Today, it is known that more than 250 diseases are caused by micro-organisms, such as pathogenic bacteria, viruses and parasites, which are ingested through food and drinking water. Bacteria have the largest part (91%) of these disease-causing micro-organisms. Because of the limited availability of useable water resources in the world, it has great importance to remove bacteria from water sources, especially Escherichia coli (E. coli), in order to ensure efficient use of water resources.

In this research, a method has been suggested to demonstrate the sufficiency of nanoparticles showing magnetic properties (particularly those that can be controlled by an externally applied magnetic field) for the reduction of E. coli populations in water resources. The suggested method briefly describes the fabrication of magnetic iron-oxide nanoparticles and their functionalization with biotin,streptavidin,biotin+streptavidin,biotin+streptavidin+aptamer. The study also covers reduction of E.coli bacteria and affects of nanoparticles on collecting the bacteria from water recorces. To do this Flow cytometry measurements were used.

Preliminary and detailed studies of the subject indicate that modified and/or non-modified magnetic nanoparticles have considerable potential for the reduction of E.coli populations in water samples.

Keywords: Escherichia coli, iron oxide , nanoparticle, streptavidin, biotin, aptamer

SYMBOLS

K_d **:** Decomposition constant

K_B **:** Boltzmann constant

K **:** Anisotropy constant

N-n **:** n-aminohexyl

t **:** time (s)

T **:** Temperature (°C)

V **:** Particle size as the volume

r **:** Particle size

T_g **:** Glass transition Temperature (°C)

H_C **:** Coercivity change

Da **:** Dalton

Psi **:** Pounds by the square inch

pH **:** Power of hydrogen

O **:** Oxygen

H **:** Hydrogen

CO_2 **:** Carbon dioxide

NaOH **:** Sodium Hydroxide

FeCl **:** iron chloride

FeO **:** iron oxide

Fe(III)Cl **:** Ferric chloride

ABBREVIATIONS

NT **:** Nanotechnology

NP **:** Nanoparticle

E.coli **:** Escherichia coli

DNA **:** Deoxyribonucleic acid

RNA **:** Ribonucleic acid

SS **:** Single-stranded

EA **:** Electron Affinity

SPM **:** Superparamagnetic

UV **:** ultraviolet

SELEX : Sequential Evolution of Ligands with Exponential Enrichment

PBS **:** Reduction Buffer Solution

NHS **:** N-Hydroxysuccinimide

PCR **:** Polymerase Chain Reaction

HDT **:** temperature under heat

SPM : superparamagnetic

FSC **:** Forward Scatter channel Detector

SSC : Side scatter channel detector

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1. INTRODUCTION

1.1. Introduction

Nanomaterials are particles that are less than 100 nanometers (nm) and are much more efficient in terms of ease of use, more space-saving, cheaper and more suitable for new functions. Due to this practicality of nanoparticles, they are used in many industrial fields such as electronics, biomedical, automotive and chemical sectors. This is reflected in the accelerated studies on nanowires within the last 10 years.Exemplary structures are nanowires; nanocrystals, nanoparticles, nanotubes, nanowires, nanorods or nano-thin films.

The fabrication of nanomaterials has led to the development of many technological and pharmacological products such as high activity catalysts, special conductive materials for optical applications, superconductors, anti-wear additives, surfactants, drug carriers and special diagnostic tools. In addition, the control of the materials at the nanoscale level allows the realization of miniaturized devices with unique functionality, such as sensors and high-density data storage cells.

Food-borne diseases caused by diarrheal E. Coli have clinical, public health and economic importance. The costs of treatment and loss of labour caused by E. coli O157: H7 serotype are estimated to be the US $ 229-610 million per year[1].

The nanoparticles are much more efficient in terms of being light, compact, inexpensive, and more suitable for every application. Molecular analyses of various epidemics and sporadic cases in the world have shown that strains of different genotypes are responsible for the disease spreading not only in one strain but in the whole world. In light of this information, it is very important to know which strain belongs to the sample from the source of E.coli. Fast and effective detection of aptamers from different E.coli strains can be achieved by using suitable chemicals for the nanoparticle.

As a result of the literature research, the most suitable chemical adhesives were found to be streptavidin and biotin.

Figure 1.1. Streptavidin-biotin binding sites diagram.

Streptavidin diagram is given in Figure 1.1 and it has 3 binding sites for biotin and one binding site for an iron oxide nanoparticle.

As is known, the avidin-biotin complex has the most non-covalent interaction between a protein and its ligand. The decomposition constant (Kd) between avidin and biotin is 10^{-15} M,

and the avidin-biotin complex is formed in a very short time and the pH change is not affected by high temperature, organic solvents and other denaturing factors. Because of these properties, the avidin-biotin system is used for immobilization and marking in many bioanalytical applications. In the General Information section, there will be more information about streptavidin and biotin.

Nucleic acid aptamers are small, single-chain DNA or RNA oligonucleotides with stable, three-dimensional, sequence-dependent structures. Due to their high specificity binding to the targets of nucleic acid aptamers, very small structural differences of target molecules can be distinguished.

Figure 1.2 Streptavidin and biotinylated enzyme complex diagram

As seen in Figure 1.2 biotinylated aptamers are bound to the streptavidin-coated surface. The aptamers were attached to the bacteria and then the magnetic field was applied to collect the particles. The aim of this thesis is the fabrication of nanoparticles and use them as a transducer to collect E.coli bacteria from water sources. Escherichia coli is the most frequently observed species among facultative anaerobic bacteria in the gastrointestinal tract. Because genetic material is frequently used, it is shown as a model organism in learning E. coli surface colonization. They create a biofilm on the water pipes, in water systems and adversely affect public health.

1.2. Purpose and Importance

In this master thesis; iron oxide nanoparticles were prepared using materials such as $FeCl_3.6H_2O$, D-Glucose($C_6H_{12}O_6$), Sodium hydroxide(NaOH) and distilled water by the hydrothermal and high-temperature method. The purpose of the study was given below;

- To detect whether or not iron oxide nanoparticle capture E.coli bacteria by using chemical adhesives (streptavidin and biotin)
- Investigating the success of the binding bacteria in doubling water samples in which the iron oxide nanoparticles are kept constant.
- To detect whether or not aptamer attached on nanoparticles (by using chemical adhesives streptavidin and biotin)
- Accurate determination of the appropriate amount of nanoparticle-streptavidin-biotin and aptamer
- Detection of the time required for the chemical bonding of the aptamer to the nanoparticle

The iron oxide nanoparticles prepared for researching the above-mentioned properties were subjected to various processes. These iron oxide nanoparticles are collected from water samples contaminated with E. coli with the help of a magnet (4000 Gauss).The numbers of dead and injured live organisms were determined by flow cytometry device. The functionalization of the iron oxide nanoparticles by combining them with the so-called Aptamer-specific constructs for bacterial DNA using chemical adhesives such as streptavidin and biotin has been studied very little. In order to be able to use the iron oxide nanoparticles in different applications and to increase their efficiency in their current applications, it is important to determine the preparation of these particles, the conditions of preparation and the effect of addition of atoms on their structural and magnetic properties for the functionalization of iron oxide nanoparticles.

2. GENERAL INFORMATION

2.1. The Magnetic Iron Oxide Nanoparticles

Iron oxides (FeO), Fe ions and O^{-2} or OH^{-} ions are formed by regulation. Although the diameter of the O^{-2} ion is 0.14nm, Fe^{+2} and Fe^{+3} ions are 0.065nm and 0.082nm respectively, it is clear that the sequence of the anions is the factor of the topological transformations between the crystal structure and the different iron oxides. There are two basic pathways showing the structure of iron oxides; They are either tetrahedral or octahedral connections consisting of either anion-cation and the closest neighbouring anions to regulate the anions (packaging). Fe_3O_4, known as magnetite, has an inverse spinel crystal structure with cubic unit cell centre shape where oxygen ions are placed in the cube in positions corresponding to each other along three axes. The unit cell consists of 56 atoms, including 32 O^{-2} anions, 16 Fe^{+3} cations and 8 Fe^{+2} cations[2].

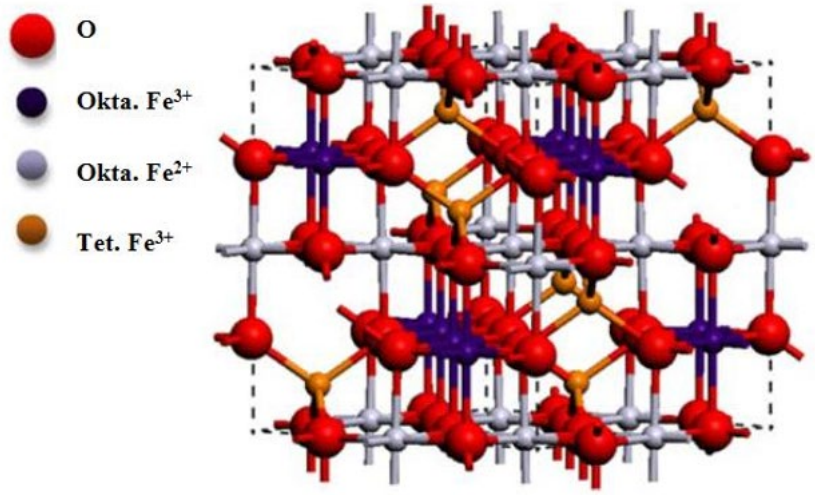


Figure 2.1 Fe_3O_4 has an inverse spinel crystal structure with a cubic unit cell [3].

Iron oxides are found in various forms in nature. The most common forms are magnetite, (Fe_3O_4), maghemite (γ-Fe_2O_3) and hematite (α-Fe_2O_3). Among these magnetites Fe_3O_4, has the strongest magnetism due to its more stable structure. Stable iron oxides are extremely stable under favourable conditions, and under certain conditions, the iron oxides are transformed into each other.

2.1.1. Magnetic Properties of Iron Oxide Nanoparticles

As is known, the magnetic properties of Fe_3O_4 nanostructures are quite different from the bulk Fe_3O_4. In the single domain nanoparticle, only magnetization rotation occurs, whereas, in bulk material, magnetization increases with sensitivity to the field through field motion until rotation. In general, the magnetic behaviour of nano-crystals depends on the physical properties of each particle.

The most important physical properties include chemical composition, particle size, particle morphology, intrinsic material parameters (magneto crystal anisotropy, saturation magnetization, etc.), surface/interface and particle size distribution [3]. For the single domain particle, the energy required to overcome the energy barrier of the inverse magnetization from a stable magnetic configuration to another is given by the ratio KV / k_B T. Where K is the anisotropy constant of the particle, V is the particle size, the k_B Boltzmann constant and the T temperature. Thus, if the particle size is reduced sufficiently, it is possible for the thermal energy to exceed the anisotropic energy.

2.1.2. Synthesis of Nanoparticles by the Hydrothermal and High-temperature Method

Hydrothermal synthesis of Fe_3O_4 nanoparticles has been reported in the literature. Hydrothermal reactions are carried out in the reactors or in aqueous media in autoclaves where the pressure is greater than 2000 psi and the temperature can rise above 200 ° C. There are two main ways for the formation of ferrites by hydrothermal conditions: hydrolysis and oxidation or neutralization of mixed metal hydroxides. These two reactions are very similar but iron salts are used in the first method.

In this process, variables such as solvent, temperature and time have significant effects on the reaction and products. It has been observed that the particle size of the Magnetic iron oxide (Fe_3O_4) powders increased with the reaction time and more water addition caused the precipitation of larger (Fe_3O_4) particles. In the hydrothermal process, the particle size in the crystallization is controlled primarily by adjusting the ratio of the material used and the applied temperature level. The nucleation and grain growth processes depend on the reaction temperature when other conditions are kept constant.

Figure 2.2 Crossectional view and components of the autoclave.

2.2. Biological Factors Used in this Research

2.2.1. Escherichia Coli Bacteria

The bacterial isolate is Escherichia coli. E. coli Gram-negative 1.1-1.5 x 2.0-6.0, µm dimensions of the boom-shaped, non-spore, moving, facultative anaerobic bacteria. There are still strains. Nutrient agar, viscous and selective media of blood agar bacteria form visible colonies in the 24-hour period. The bacterial isolate Escherichia coli obtained from the Department of Microbiology at Cerrahpaşa university [4].

2.2.2. DNA

Nucleic acid aptamers are small, single-chain DNA oligonucleotides with stable, three-dimensional, sequence-dependent structures. Due to their high specificity binding to the targets of nucleic acid aptamers, very small structural differences of target molecules can be distinguished.

i. Deoxyribose Nucleic Acid - DNA

DNA consists of two simple unit polymers called nucleotides. These polymers consist of sugar and phosphate groups linked together by ester bonds. Each sugar group is bound to one of four types of molecules called bases. These bases of DNA encode genetic information. Multiple nucleotides are bound together to polynucleotides [5]. The hydrogen bonds between the double-stranded and two-stranded base stabilize the DNA. The four bases in DNA are called adenine (abbreviated A), cytosine (C), guanine (G) and thymine (T). These four bases bind to sugar phosphate to form a nucleotide, for example, "adenosine monophosphate" is a nucleotide.

ii. 3' - 5 'of the DNA (Deoxyribose Nucleic Acid)

The DNA strand is composed of sugar and phosphate residues [6]. The sugar molecules are inside of the DNA is 2-deoxyribose, which is a pentose (five-carbon sugar). The phosphate group between the third carbon of one of the two adjacent sugars and the carbon atom fifth of the other form a phosphodiester bond and bind the sugars together. The phosphodiester bond is asymmetric thats why the DNA strand has one aspect. In the double helix, the direction of binding of nucleotides to one another is the inverse of the direction of the other strand. This pattern of DNA strands is called antiparallel. The asymmetric ends of the DNA strands are called 5 '(five bases) and 3' (three bases), the 5 'end carries a phosphate group and the 3' end carries a hydroxyl group.

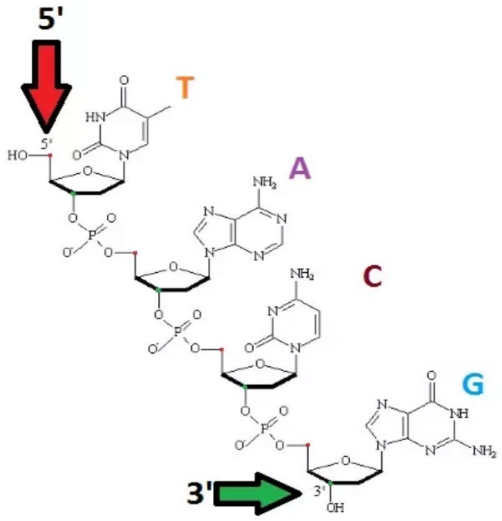


Figure 2.3 Structure 3' - 5 'of the DNA

2.2.3. Aptamer

Aptamers are a type of nucleic acid regulated in the laboratory and are generated by the method of SELEX (systematic evolution of exponential enrichment) [7]. Aptamers are single strained DNA or RNA (ssDNA or ssRNA) molecules. Aptamers are oligonucleotide or peptide molecules that target binding to a particular molecule. Aptamers are synthesized in laboratories by selecting from a large random sequence pool.

i. SELEX Aptamer Selection

SELEX is an acronym of systematic evolution of ligands by exponential enrichment. This method consists of an iterative process. The starting library is amplified by PCR (Polymerase Chain Reaction) and transcribed into RNA. PCR is a common name given to the reactions that are applied in order to enzymatically amplify a specific region between two segments known in the DNA. The target-specific RNA is determined by association with the target molecule, and the tightly bound RNA molecule is transcribed from the medium and reverse transcribed into the cDNA. Next, double helix DNA is amplified by PCR. These enriched binding sequences are again transcribed into RNA. This operation is repeated between 5 and 12 and the most closely bound RNA to the target molecule is selected. This procedure was applied in the department of microbiology at Cerrahpaşa University.

2.3. Chemicals That Bind Aptamer to the Nanoparticles

As is known, the avidin-biotin complex has the most non-covalent interaction between a protein and its ligand. The decomposition constant (Kd) between avidin and biotin is 10^{-15} M, and the avidin-biotin complex is formed in a very short time and the pH change is not affected by high temperature, organic solvents and other denaturing factors [8]. Because of these properties, the avidin-biotin system is used for immobilization and marking in many bioanalytical applications.

2.3.1. Avidin and Streptavidin

Avidin, a 67 kDa glycoprotein, contains the same subunit in four of the 128 amino acids [9]. It has been shown that the avidin gene can be cloned from chicken eggs [10]. Avidin, a biologically active recombinant (ie, by cutting off its molecules by genetic engineering technology and by combining the different fragments obtained), is the gene cloned from Escherichia coli.

A 52.8 kDa protein synthesized from reduction Streptomyces avidiniiii bacteria is streptavidin. It has a high affinity for biotin, streptavidin homo tetramers. Homotetramer is a protein complex consisting of four identical subunits. These subunits are unified but don't have a covalently connected structure.

2.3.2. Biotin

Biotin consists of an ureido ring joined by a tetrahydrothiophene ring. Tetrahydrothiophene is an organosulfur compound with the formula $(CH_2)_4S$. It has a five-membered ring composed of four carbon atoms and a sulfur atom. It is the saturated analogue of thiophene. It has an unpleasant odour and it is volatile, colourless liquid. Thien, thiolane or THT are also present in the names of biotin. It has the task of hosting carbon dioxide, ureido ring in the carboxylation reactions. A valeric acid (Valeric acid or pentanoic acid, CH3 (CH2) 3COOH. A straight-chain alkyl carboxylic acid having that chemical formula. It is added to one of the carbon atoms of the tetrahydrothiophene ring. Biotin synthesis diagram has been given below.

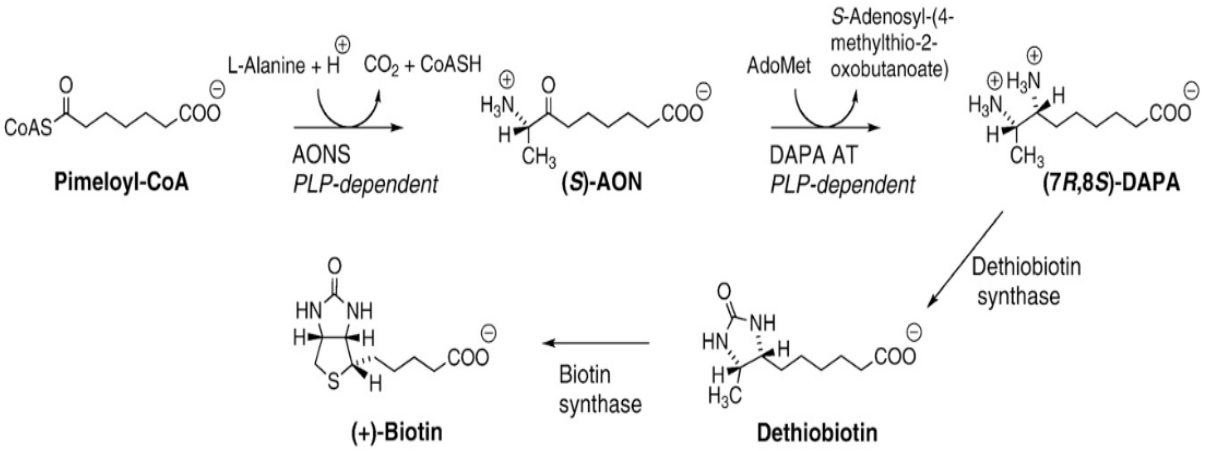


Figure 2.4 Biotin Synthesis Diagram

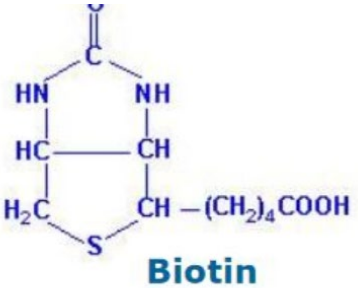


Figure 2.5 Structural Formula of Biotin

2.3.3. Biotin-Streptavidin Interaction

First, there is a high shape unison between the binder pocket and biotin. Secondly, there is a large hydrogen bonds formed in the connecting area of biotin. There are eight hydrogen bonds directly to the residues in the bonding zone (called the "first shell" of the hydrogen bond), which includes Asn23, Tyr43, Ser27, Ser45, Asn49, Ser88, Thr90 and Asp128 residues. There is also a "second hydrogen binding shell" that have the remnants that interact with the remains of the first shell. The Biotin clamping pocket is hydrophobic and there are numerous van der Waals force-mediated bindings and biotin hydrophobia interactions that are thought to be formed by high affinity in the pocket. In particular, the pocket is covered with remnants of tryptophan preserved. Finally, the ratio of connecting the biotin to a flexible loop connecting the B strip 3 and 4 (L3/4), which acts as a ' lid ' on the binding pocket and contributes to extremely slow biotin separation, closing on the connected biotin [12], [13], [14].

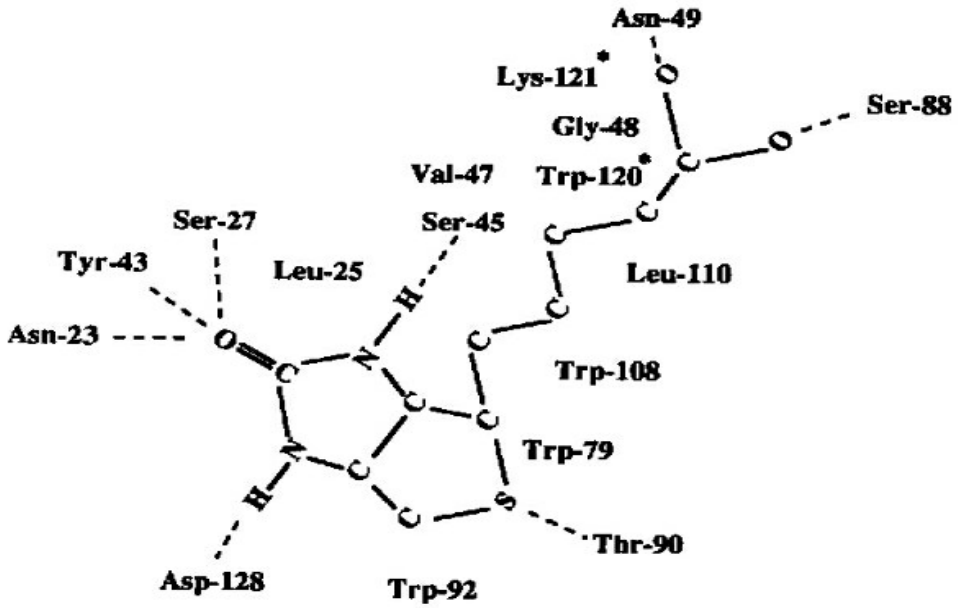


Figure 2.6 Biotin – Streptavdin Interaction

2.4. Devices Used in this Research

2.4.1. Flow Cytometry

Flow cytometry is a device that measures the number of cells (dead/live/injured) in a flowing fluid. Cells or particles in suspension with flow cytometry are passed through a chamber illuminated by laser light; When the cells pass through the light, the signals that they give are analyzed. The source of the signals formed by the physical properties of the cell, such as size and granularity; various fluorochromes which bind to the cell. When each cell or particle passes through the laser beam, the fluorescent light emitted by the deflected laser beam cells. They are transformed into analogue signals with different wavelengths by optical filters and mirrors. These signals are digitized and histograms are generated. The histograms are the visual representation of the frequency distributions of the measured parameters.

Flow cytometry can analyze the properties of cells in four ways.

- By measuring the size of the cell and by determining the granule structure of the cells
- By analysing the fluorescent properties of the cell.
- By analysing antibodies marked with multicoloured fluorescent dyes (2-17 colours)
- By analysing fluorescent dye-labelled antibodies added by increasing cell permeability.

2.4.1.1. Fluid System of the Flow Cytometry

The fluid containing bacteria filled the device through the channel. The fluid containing the sample in the centre (laminar flow) and the surrounding fluid (sheath fluid) loads without mixing. With this effect, the particles are arranged as a single secret and this process is called hydrodynamic focus. The fluid system is used to carry the cells through the laser beam in a single row. Thus, the laser beam focused one time on to every single cell and in this way individual cells are analyzed.

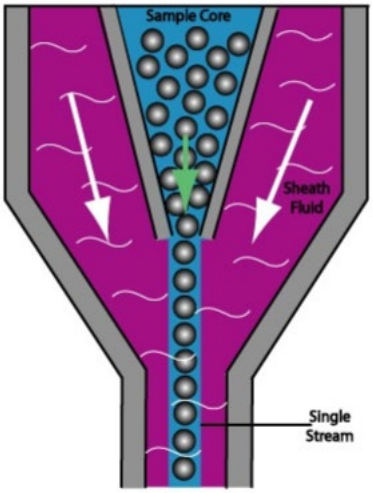


Figure 2.7 Fluid System of the Flow cytometry [15], [16].

2.4.1.2. Light Source of the Flow Cytometry

As a light source, xenon and xenon-mercury lamps can be used in the flow cytometer device, but often argon and helium laser sources are used in addition to different types of lasers. Two events occur when the cells pass in front of the laser beam. The first event is the spread of the rays according to the size and structure of the cell. The second event is the diffusion of rays into the fluorochromes taking place in/on the stained cell.

Fluorescent antibodies are called fluorochrome. Fluorochromes absorb energy from the laser beam and reflect them from different wavelengths. Rays of different reflected wavelengths are collected through detectors and transferred to the computer system in the form of available information. Fluorochrome-labelled antibodies are specific cell surface receptors or intracellular proteins used for the detection of different groups of cells.

In desktop flow cytometry systems, argon laser is usually used and fluorescent dyes such as FITC (fluorescein isothiocyanate), PE (phycoerythrin) can be activated at 488nm and allow for fluorescence intensity measurement for the cell.

2.4.1.3. The Electronic System of the Flow Cytometry

i. Forward Scatter Channel (FSC) Detector

When the beam is spread forward, it is collected with the help of the FSC lens.
The FSC detector gives information about the size of the cell.

ii. Side Scatter Channel (SS4C) Detector

The measurement of the beam at a 90-degree angle to the predetermined axis line is called the side scatter. SSC gives information about the internal structure of the particles.

iii. Fluorescence Filters and Detectors

Fluorescence measurements at different wavelengths give molecular information, such as cytokines and DNA, with fluorochrome-labelled cell surface receptors.

2.4.1.4. Steps for the Flow Cytometry

1. Preparation of cell suspension
2. Antibody marking of cells
3. Device Calibration
4. Choosing fluorochromes for marking of antigens
5. Data analysis and reporting

1. Preparation of Cell Suspension

During the measurement, attention was paid not to aggregate the cells. Quite small volumes of cell suspension, such as fewer than 103 µl, are enough for flow cytometry measurements. The centrifuge was used during the preparation. The antibody was used at the concentration indicated by the manufacturer for the cell amount.

2. Marking of Cells with Antibodies

Bacteria are too small to give strong fluorescent molecules. Therefore, the cells were labelled with effective fluorescent emitting chemical dyes. The fluorescence of the antibody was measured, in order to determine the number of cells (dead/live/injured) in the sample solution.

3.Calibration of the Device

Fluorescent-labelled beads were used to control the optical sensitivity and linearity of the device. There was no deviation in light scattering and fluorescence measurements. It was also calibrated at bacterial concentration.

4.Choosing Fluorochromes for Marking of Antigens

DNA staining with a fluorochrome in propidium iodide (PI) was performed to distinguish live and dead cells from the whole population, and Fluorescein isothiocyanate (FITC) was preferred, which had the lowest fluorochrome molecular weight to mark the intracellular antigens.

5.Analysis and Reporting of Data

Obtained data can be shown with different ways.

1.Dot space graphics
2.Histogram
3. District Show
4.Gating

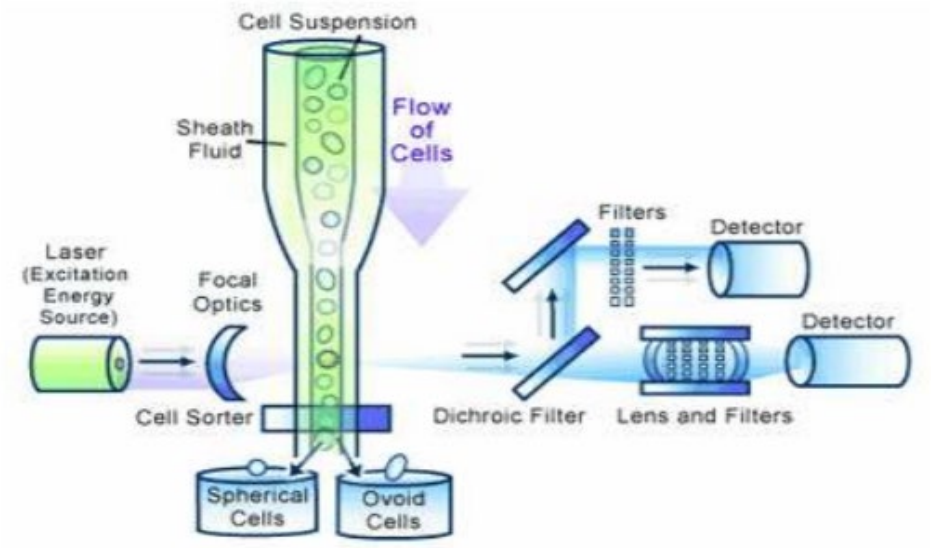


Figure 2.8 Working Principle of Flow Cytometry [15], [16].

3. EXPERIMENTAL STUDIES

Experimental studies of the thesis contain main parts are given below;

i. Synthesis of Iron oxide (FeO) Nanoparticles

- Weighing the materials required to syntheses nanoparticles,
- Preparation of the solution consisting of weighed and mixed materials in an autoclave according to the hydrothermal and high-temperature method,
- Drying of iron oxide nanoparticles,

ii. Functioning Ironoxide Nanoparticle with Aptamer

- Separation of nanoparticles into groups and coating with streptavidin
- Coating of streptavidin-coated nanoparticles with biotin solution,
- Fixation of the aptamer to iron oxide nanoparticles coated with streptavidin-biotin.

iii. Collecting E.coli bacteria from samples

- Preparation of E. coli solution,
- Collecting E.coli bacteria from samples with Aptamer-functionalized iron oxide nanoparticles by using a magnet (4000 Gauss)
- Preparation of E.coli solution to flow cytometry measurements,
- Staining bacteria for flow cytometry measurement,
- Placing samples in a flow cytometry device and analysis of outputs (graphs and tables) of the device,

3.1. Used Bacterial Isolate

The bacterial isolate Escherichia coli (ATCC25922) obtained from the Department of Microbiology at Cerrahpaşa university. [4]. In order to observe the results of the process by spreading to the solid media, the bacterial isolate Escherichia E.coli (ATCC25922) was diluted to 1/1 000 000 concentration, according to the instructions In this research, the magnetic field was applied to the Escherichia E.coli (ATCC25922) solution.

3.2. Starting Material Ferric Chloride ($FeCl_3.6H_2O$) Used in the Construction of Nanoparticles

It is also called iron (III) chloride. The formula of the chemical compound is FeCl3. The anhydrous compound is a crystalline solid with a melting point of 307.6 ° C.The colour of chloride (III) crystals depends on the angle of view with reflected light. The crystals appear dark green, but the sent light appears purple-red. Dehydrated iron (III) chloride is soluble in hydrated hydrogen chloride mist formed in the humid air.

When dissolved in water, iron (III) chloride is exposed to hydrolysis and gives an exothermic reaction temperature. The resulting brown acidic and abrasive solution is used as a coagulator in wastewater treatment and drinking water production, and printed circuit boards are like copper-based metals paint. Dehydrated iron (III) chloride is a very strong Lewis acid and is used as a catalyst in organic synthesis [17],[18].

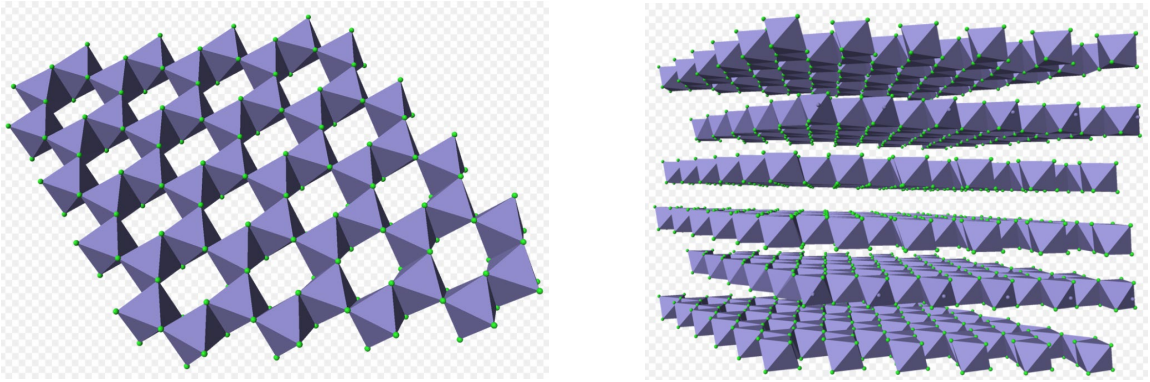


Figure 3.1 Iron (III) Chloride structure a top view b side view

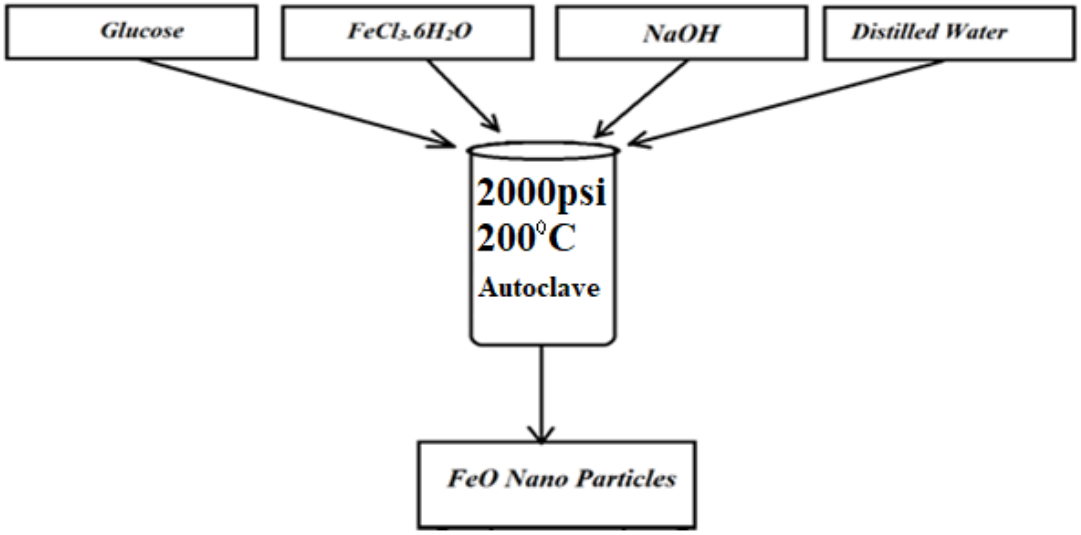


Figure 3.2 Synthesis Diagram for Iron oxide Nanoparticle

3.3. Synthesis of Iron Oxide(FeO) Nanoparticles

To produce nanoparticles, D-glucose ($C_6H_{12}O_6$), Ferric(III)chloride ($FeCl_3.6H_2O$), sodium hydroxide (NaOH) were all analytical grade and used without further purification [17]. The procedure for the synthesis of magnetite iron oxide nanomaterials are described as follows: First, 1,35 g of $FeCl_3.6H_2O$ and 3.6 g of glucose were dissolved in 30 mL of distilled water and then 0.1 mol of the precipitating agent (NaOH) was added under vigorous stirring for 30 minutes approximately.

The D-glucose ($C_6H_{12}O_6$), Ferric chloride ($FeCl_3$ $6H_2O$), sodium hydroxide (NaOH) mixture was then transferred to a 50 mL stainless steel autoclave, sealed and kept at 160°C for 6 hours.

After 6 hours in an autoclave, the reaction system was naturally cooled to room temperature, the solid products were separated by a magnetic field, washed with distilled water and ethanol, and dried in vacuum (10 mbar) at 60 °C for 10 hours.[19] SEM (Scanning Electron Microscope) images of the synthesized particles were presented in Fig.3.3.

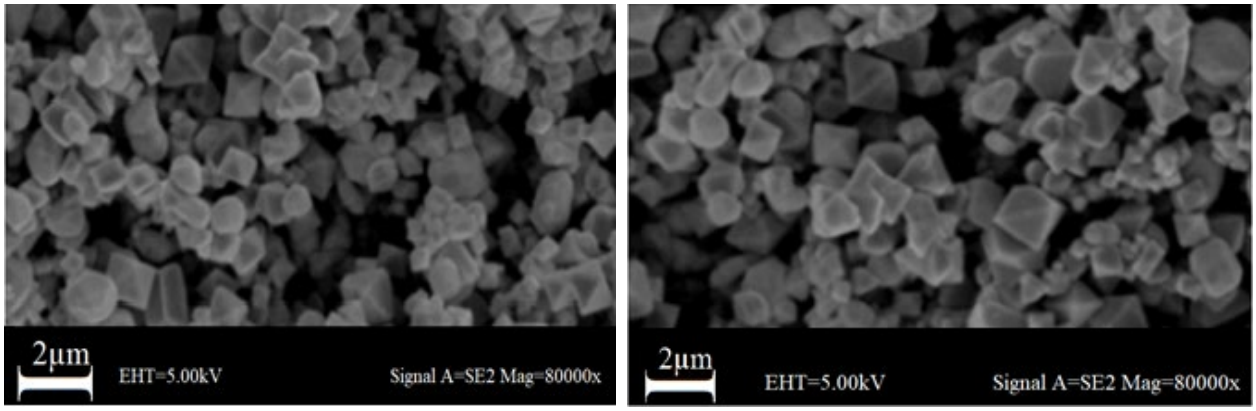


Figure 3.3 SEM images of synthesized iron oxide nanoparticles

3.4. Functioning Iron Oxide Nanoparticles with Aptamer

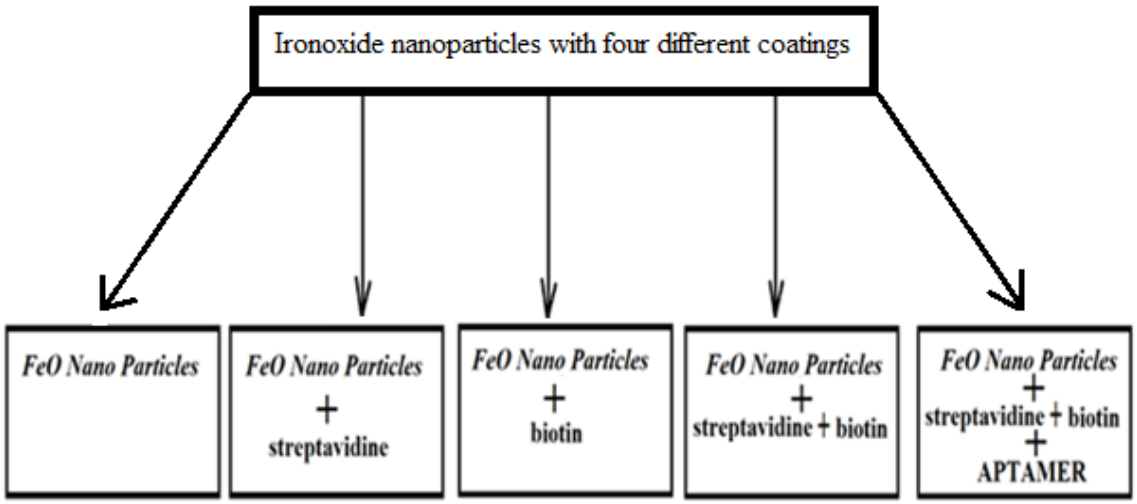


Figure 3.4 Iron oxide nanoparticles with four different coatings

In order to compare the efficiency of collecting E.coli bacteria of the structures, given in the diagram in Figure 3.4, iron oxide nanoparticles were coated with different materials (streptavidin, biotin,streptavidin+biotin, streptavidin+biotin+aptamer). The nanoparticles were divided into groups of 0.1 grams. Five types of iron oxide nanoparticles were prepared given ;

i. Non-treated iron oxide nanoparticles: Fabricated nanoparticles have a ferrite structure. In the hydrothermal treatment of the ferrites, the particle size in the crystallization is mainly controlled by the proportion of the used material and adjusting the applied temperature level.

ii. Streptavidin-coated iron oxide nanoparticles: Streptavidin dissolved in 1,4-phenylene diisothiocyanate, SiO_2 and NH_2 added Tris-HCl buffer, streptavidin was added dropwise on iron oxide nanoparticles and dried at 25 ° C.

iii. Biotin coated iron oxide nanoparticles: Biotin powder was mixed with water in a ratio of 1: 2.
iv. Streptavidin+Biotin coated nanoparticles: Ironoxide nanoparticles were coated with streptavidin. The prepared biotin solution was then dropped and dried to the coated nanoparticles.
v. Streptavidin+Biotin +Aptamer coated Ironoxide Nanoparticles: 1 mg/ml biotin solution was prepared in saline and modified aptamer was added and allowed to stand for one hour. Modified aptamer (specially prepared for E.coli type) is taken from Cerrahpaşa University Microbiology Department. Then, a biotin-coated modified aptamer solution was dropped on the streptavidin and biotin-coated iron oxide nanoparticle and left to stand for 1 hour.

3.5. Collecting E.Coli Bacteria from Samples

E.coli bacteria obtained from Cerrahpaşa University were dissolved in saline solution and mixed with a magnetic stirrer. The most reliable way to create approximately the same amount of bacteria in each solution is to prepare a bacterial solution in the same absorbance. Therefore, the absorbance of the bacterial solution was measured by using Shimadzu UV-visible spectrophotometer (model: UV-1280). The E.coli solution was concentrated at a wavelength of 625 nm until the absorbance was 0.13. Bacterias are collected by the functionalized iron oxide nanoparticles. To do this, the magnetic field is applied (4000 Gauss) as seen in Figure 3.5 for 45 minutes towards the saline solution with bacteria and functionalized iron oxide nanoparticles.

Figure 3.5 Collecting Nanoparticles with Magnet

3.6. Preparation of E.Coli Solution for Flow Cytometry Measurements

As seen in Figure 3.6, iron oxide nanoparticles that capturing bacteria from samples. After capturing bacteria from samples it is necessary to stain leftover bacteria in solutions for flow cytometry measurement. A kit was used for this staining process. Preparation of the final

staining concentrations to be 420 nmol / L for TO and 43 nmol / L for PI. The prepared solution was placed into the flow cytometry device. The data were analyzed after obtained from the device in the form of graphs like the dot space graph.

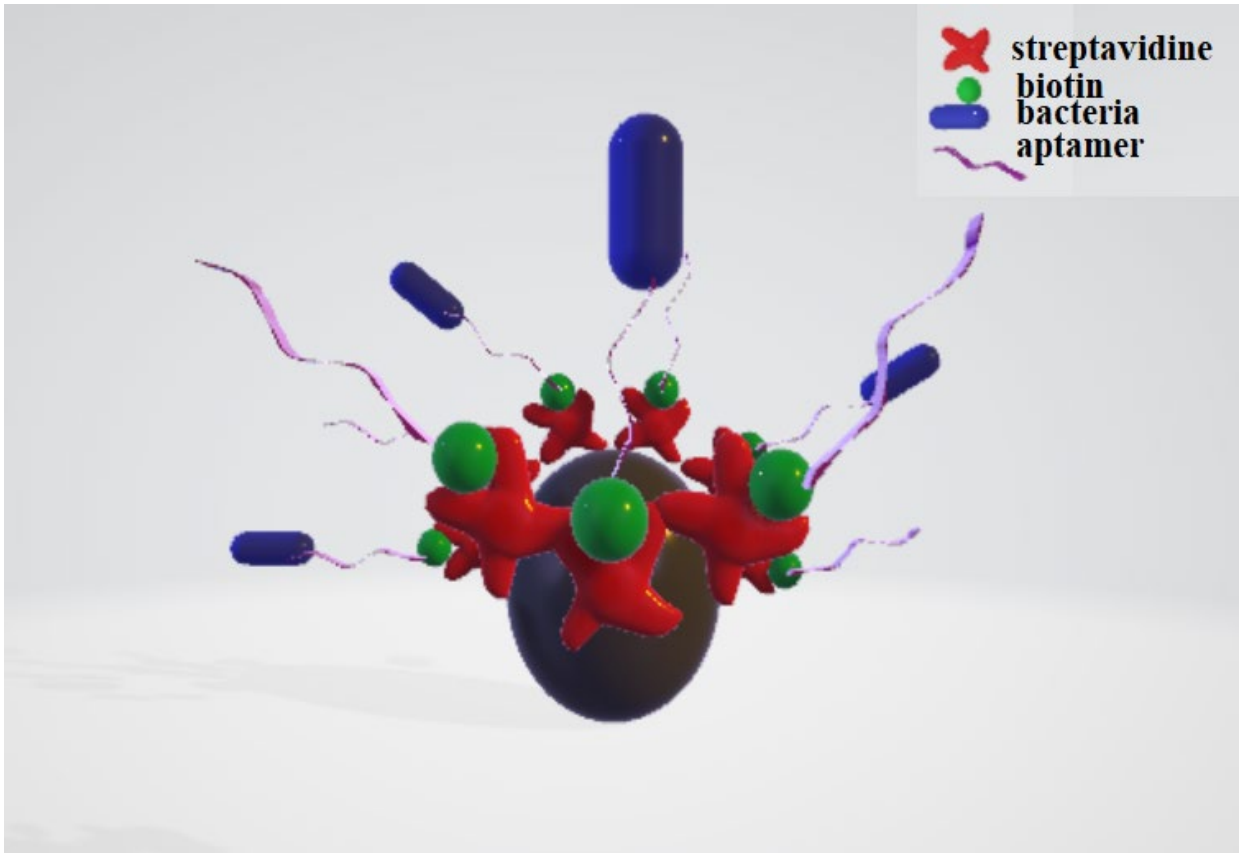


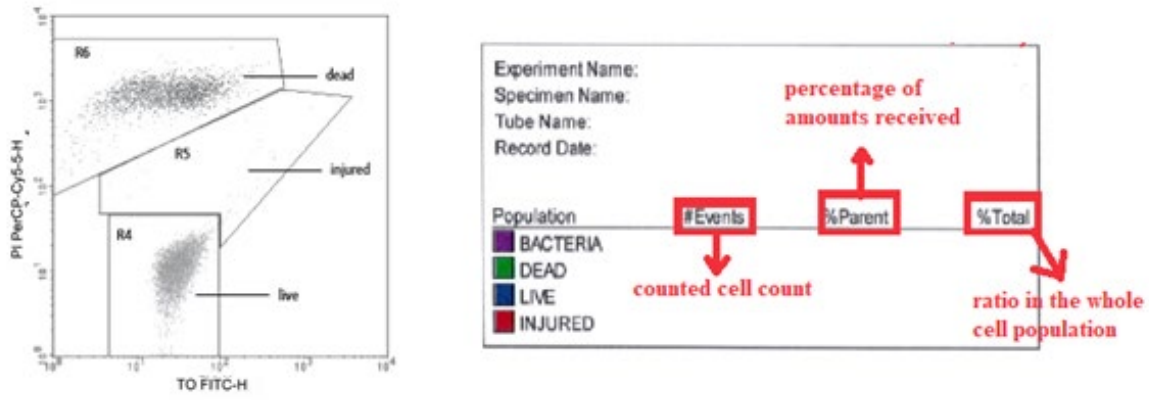
Figure 3.6 Bacteria capturing diagram of iron oxide nanoparticles

3.6.1. Flow Cytometry Measurements

BD™ Cell Viability Kit was used in the determination of cell concentration. Flow cytometry counts viable cells in eukaryotic and prokaryotic cell suspensions. [20-21-22-23] Thiazole orange (TO) solution to stain all cells and propidium iodide (PI) to stain dead cells. BD Liquid Counting Beads is a liquid suspension of fluorescent beads.

PI dyes dead cells membranes and are impermeable. TO is a permeant dye molecule it moves in to the cell and enters into all cells, live and dead.

In order to stain the bacteria in the solution, 500 µL of dye solution were used for each 5.0 µL of bacteria solution. Each dye solution contains 420 nmol/L for Tiozole Orange (TO) and 43 µmol/L for propidium iodide (PI). [24-25]



a **b**

Figure 3.7 Outputs of the flow cytometry device (a) Dot space graphs, (b) Diagram

Typical outputs of the flow cytometry device were given in Figure3.7. Dot-space graph (Fig.3.7.a) gives information about the number of live/dead/injured cells. Live cells are in Region4; injured beads are in Region5 and dead cells are in Region6. The horizontal axis indicates the logarithmic increase in FITC (fluorescein isothiocyanate) dyed bacteria light intensity while maximum excitation wavelength 493 nm, Excitation Laser Lines 488 nm, maximum Emission wavelength 525 nm. Vertical axis indicates the logarithmic increase in PI (Propidium Iodide) PerCP-Cy5-5-H dyed bacteria light intensity while maximum excitation wavelength 305-540 nm, Excitation Laser Lines 325-360-488 (nm), maximum emission wavelength 620nm.

Figure 3.7.b gives information about the same quantities as a diagram. The diagram also shows results about the counted cell, per cent of the amount received and ratio into whole cell population.

4. RESULTS

Iron oxide nanoparticles were synthesized and functionalized with Aptamer using the appropriate chemicals. When the literature was searched, it was noticed that there was not enough research on Aptamer adhesion to nanoparticles. In the present studies, it was realized that the time required for the chemical binding used in the process of the binding aptamer to the nanoparticle was not determined.

In this research, iron oxide nanoparticles were synthesized and then aptamer nanoparticles were bonded using chemicals such as streptavidin and biotin. When the water samples were doubled, the success of bacteria collection was investigated. In addition, the time required for the chemical binding of the aptamer to the nanoparticle was also determined.

The amount of aptamer-biotin mixture to be used for maximum binding of the aptamer to the nanoparticle was determined.

4.1. Effects of Type of the Binders on Number of Collecting Bacteria (Live/Dead/Injured) from the E.Coli Saline Solution

A total of 1 gram of iron oxide nanoparticles were synthesized as described in the third chapter. Iron oxide nanoparticles, which was 0.1 grams, were placed in separate beakers. Samples in each beaker were named Group A1, Group B1, Group C1 and Group D1. No treatment was applied to the iron oxide nanoparticles in Group A1. 0.1 ml biotin was dropped onto the iron oxide nanoparticles takes place in group B1 and dried at 35 °C. 0.1 ml streptavidin was dropped onto the iron oxide nanoparticles takes place in group C1 and dried at 35 °C. 0.05 ml streptavidin was dropped onto the iron oxide nanoparticles takes place in group D1 and dried at room temperature, followed by addition of 0.05 ml biotin and drying at room temperature.

Bacterial isolates from Cerrahpaşa University were dissolved in 400 ml of saline. The resulting 400 ml bacterial-saline solution was divided into five equal portions as 80 ml. Each beaker (80 ml bacterial-saline solution) and nanoparticles has labelled as Group A1, Group B1, Group C1 and Group D1.

Additionally, the same amount of bacteria containing the saline solution was prepared. The solution was used as a reference solution for a number of bacteria. The number of bacteria was determined by using flow cytometry measurements.

Table 4.1 Processed Nanoparticles

No	Group Name	Processed Nanoparticles
1	Group A1	Non-treated nanoparticles
2	Group B1	Biotin coated and dried nanoparticles
3	Group C1	Streptavidin-coated and dried nanoparticles
4	Group D1	Streptavidin-coated on the biotin-coated nanoparticles

Iron oxide nanoparticles were collected from the bacterial saline using a magnetic field. Each group was exposed to a magnetic field for 45 minutes. Before measuring bacterial viability in flow cytometry, measurements were made by bacterial cultivation method. Unfortunately, the Bacterial cultivation method could not be applied at all times because the necessary and sufficient conditions could not be met.

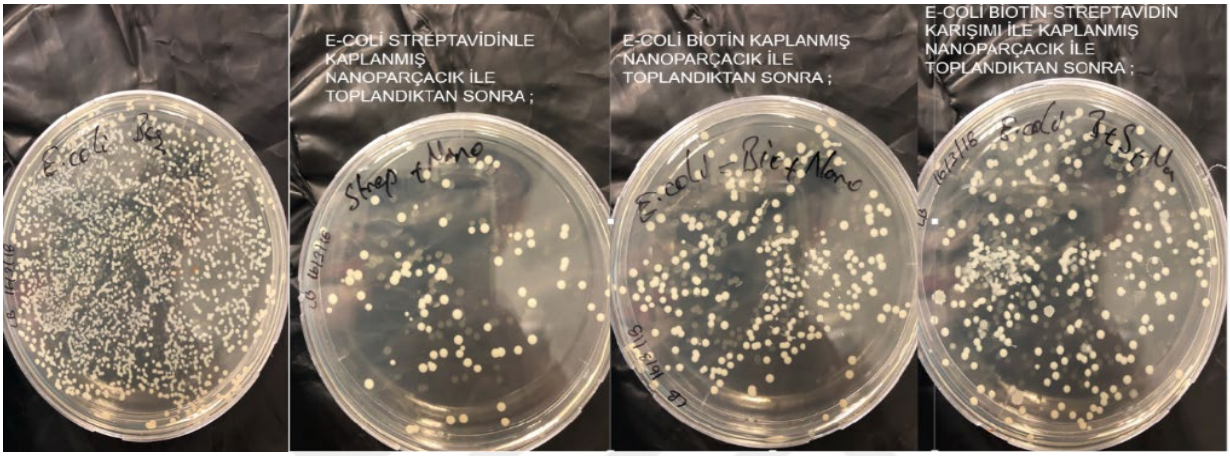


Figure 4.1 Images of the beakers.

Pictures of each beaker are as shown in Fig.4.1.When the images are examined from left to right, it is seen that the number of bacterial colonize obtained from samples of untreated E. coli bacteria solution placed with bacterial cultivation method in the first beaker has more colonies than the other beakers. The samples in the second beaker having at least E. coli bacteria belongs to the E.coli bacteria solution treated with streptavidin-coated nanoparticles.

In order to stain the bacteria in the solution, 500 µL of dye solution were used for each 5.0 µL of bacteria solution. Each dye solution contains 420 nmol/L for Tiozole Orange (TO) and 43 µmol/L for propidium iodide (PI). Flow cytometry measurements were performed after the iron oxide nanoparticles were collected from the saline solutions containing bacteria. Results of the flow cytometry measurements were obtained from the device as outputs of the device. The results were obtained as diagrams and dot-space graphs. Results of the flow cytometry measurements for this part of the study were presented in Fig.4.2. - 4.6.

Dot-space graph gives information about the number of live/dead/injured cells. The horizontal axis indicates the logarithmic increase in FITC (fluorescein isothiocyanate) dyed bacteria light intensity while maximum excitation wavelength 493 nm, Excitation Laser Lines 488 nm, maximum Emission wavelength 525 nm. And the vertical axis indicates the logarithmic increase in PI (Propidium Iodide) PerCP-Cy5-5-H dyed bacteria light intensity while maximum excitation wavelength 305-540 nm, excitation Laser Lines 325-360-488 (nm), maximum emission wavelength 620nm. The diagram also shows results about the counted cell, per cent of the amount received and ratio into whole cell population for to dead, alive and injured bacteria.

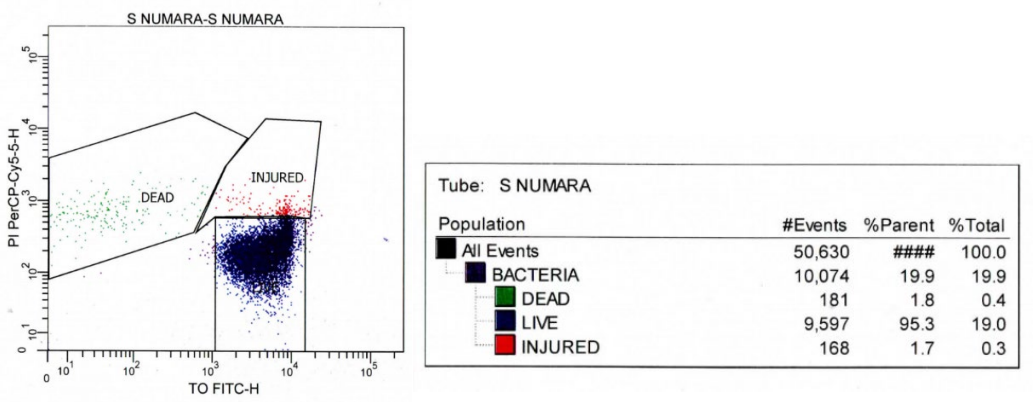


Figure 4.2 Flow cytometry measurements result for Pure E.coli solution

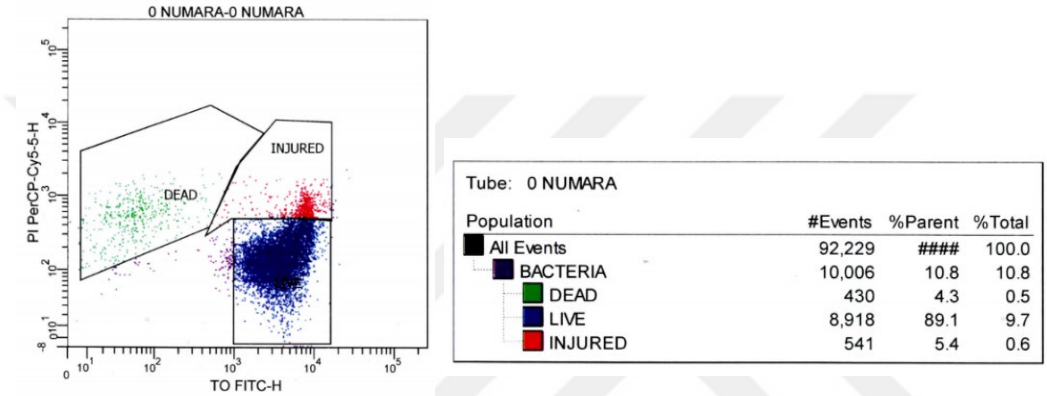


Figure 4.3 Number of bacteria live/dead/injured in E.Coli saline solution after collecting the nontreated iron oxide nanoparticles from the same solution **(**Group A1)

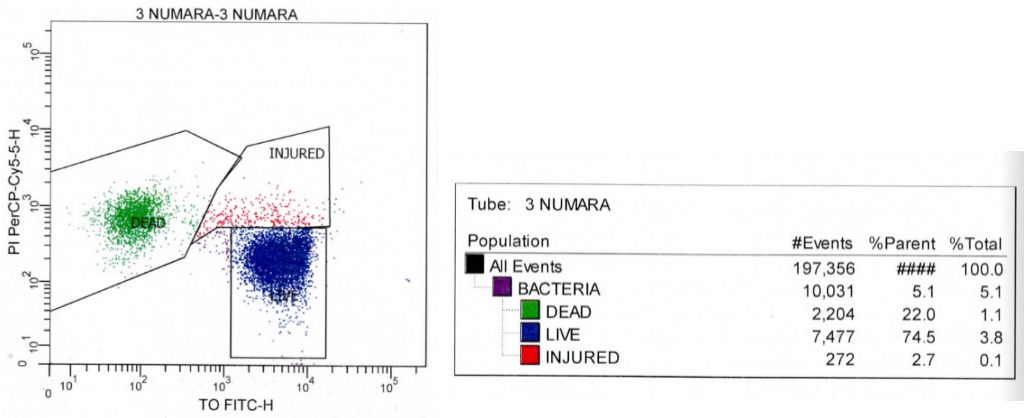


Figure 4.4 Number of bacteria live/dead/injured in E.Coli saline solution after collecting the biotin-coated iron oxide nanoparticles from the same solution (Group B1)

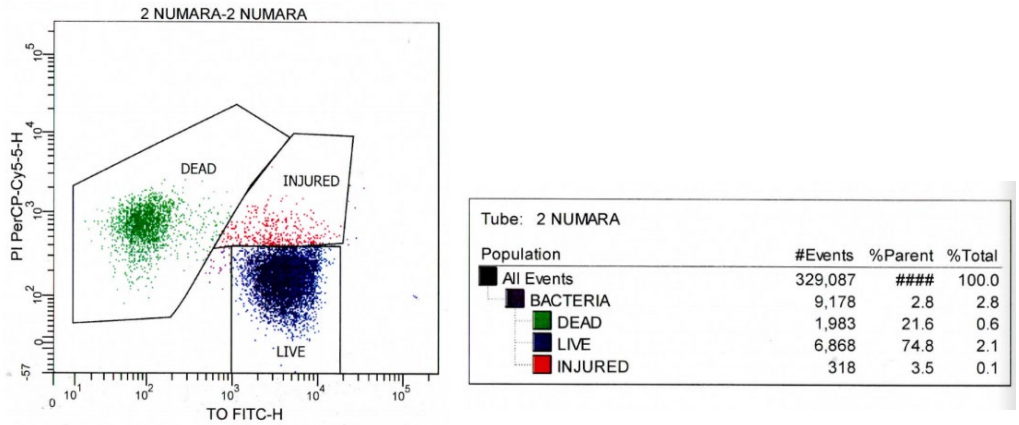


Figure 4.5 Number of bacteria live/dead/injured in E.Coli saline solution after collecting the streptavidin-coated iron oxide nanoparticles from the same solution (Group C1)

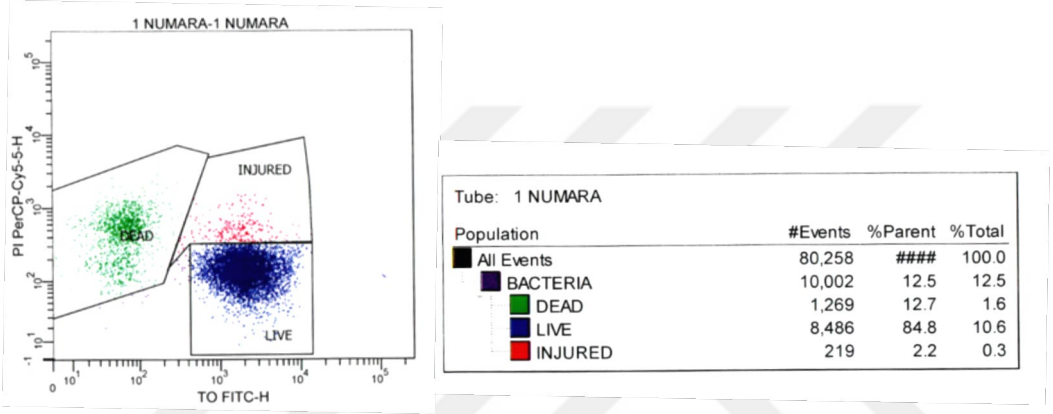


Figure 4.6 Number of bacteria live/dead/injured in E.Coli saline solution after collecting the streptavidin and biotin coated iron oxide nanoparticles from the same solution (Group D1)

Table 4.2 Effects of Type of the Binders on Number of Bacteria live/dead/injured in E.Coli saline solution after collecting the treated/nontreated iron oxide nanoparticles from E.coli saline solution

Group Names	Number of Bacteria	Number of live Bacteria	Number of dead Bacteria	Number of injured Bacteria
Pure Ecoli Solution (Group 0)	10.074	9.597	181	168
Solution After E.coli Collected by Nanoparticles. (Group A1)	10.031	7.477	2.204	272
Solution After E.coli Collected by Nanoparticles Coated with Biotin (Group B1)	10.002	8.486	1.269	219
Solution After E.coli Collected by Nanoparticles Coated with Streptavidin (Group C1)	9.178	6.868	1.983	318
Solution After E.coli Collected by Nanoparticles Coated with Streptavidin and Biotin (Group D1)	10.006	8.918	430	541

Flow cytometry measurements showed that Streptavidin was able to bind iron oxide nanoparticles to E.coli bacteria. However, when biotin was used alone, it was observed that iron oxide nanoparticles were less likely to bind E.coli bacteria than streptavidin.

As seen from table 4.2 when biotin and streptavidin used together iron oxide nanoparticles were less likely to bind E.coli bacteria than streptavidin coated nanoparticles. Biotin coated the surface of streptavidin and obstruct bacteria to hold on to streptavidin coating.

It was understood that the nanoparticles which did not undergo any treatment were able to remove some little amount of bacteria by carrying with it. As seen from the fig 4.1, the results of bacterial cultivation method have confirmed the results obtained from flow cytometry.

4.2. Effects of Volume of Bacteria-Mixed Saline on Collecting Number of Bacteria (Live/Dead/Injured) from E.Coli Saline Solution

In section 4.1, it was observed that the E.coli bacteria could be removed from the E.coli saline solution by coating streptavidin on to the iron oxide nanoparticles. It was observed that if the amount of bacterial mixed saline with the same bacteria concentration was doubled and by keeping the bacteria concentration constant, the success of nano-particles on bacterial capture was altered with respect to the solution without it is doubled.

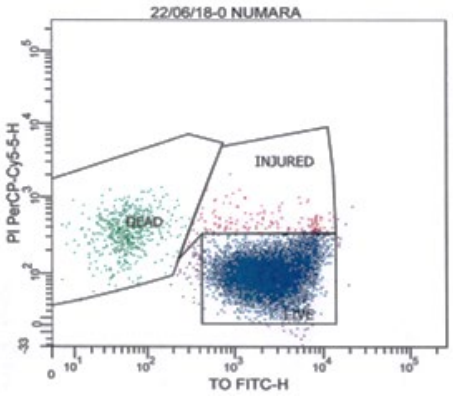
Iron oxide nanoparticles, which was 0.2 grams, were placed in separate four beakers. Each group was named Group A2, Group B2, Group C2 and Group D2. No treatment was applied to the iron oxide nanoparticles in Group A2. Iron oxide nanoparticles in Group B2 were dried at 35 ºC with 0.2 ml of biotin. Iron oxide nanoparticles in Group C2 were dried at 35 C with 0.2ml of streptavidin added. To the iron oxide nanoparticles in Group D2, 0.1 ml of streptavidin was added and dried at room temperature, followed by addition of 0.1 ml of biotin and drying at room temperature.

Table 4.3 Processed Nanoparticles

No	Group Name	Nanoparticles
1	Group A2	Non-treated nanoparticles
2	Group B2	Biotin coated and dried nanoparticles
3	Group C2	Streptavidin-coated and dried nanoparticles
4	Group D2	Streptavidin-coated on the biotin-coated nanoparticles

Bacterial isolates obtained from Cerrahpaşa University were dissolved in 800 ml saline. The obtained 800ml bacterial-saline solution was divided into five equal parts of 160 ml. 160 ml bacterial-saline solution added to four separate beads was added the iron oxide nanoparticles separated into Group A2, Group B2, Group C2 and Group D2, respectively. Iron oxide nanoparticles using magnetic field were easily collected from bacteria-mixed saline. Each group was exposed to a magnetic field for 45 minutes.

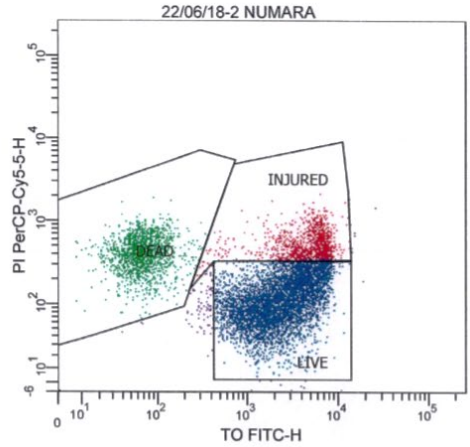
Iron oxide nanoparticles in each group were exposed to the magnetic field for 45 minutes. A kit was used to stain bacteria for flow cytometry measurement. In order to stain the bacteria in the solution, 500 µL of dye solution were used for each 5.0 µL of bacteria solution. Each dye solution contains 420 nmol/L for Tiozole Orange (TO) and 43 µmol/L for propidium iodide (PI).



Experiment Name: 22/06/18
Specimen Name: 22/06/18
Tube Name: 0 NUMARA
Record Date: Jun 23, 2018 12:17:21 PM

Population	#Events	%Parent	%Total
BACTERIA	10,000	7.4	7.4
DEAD	519	5.2	0.4
LIVE	9,249	92.5	6.9
INJURED	147	1.5	0.1

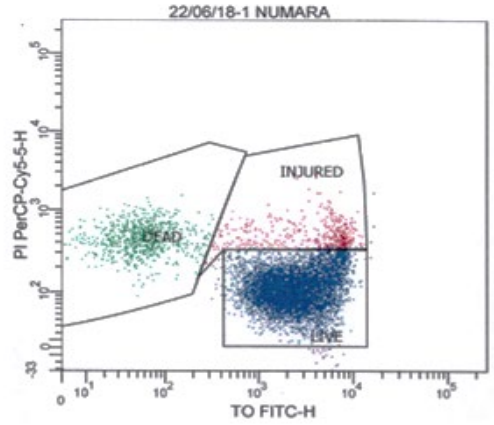
Figure 4.7 Flow cytometry measurements result for Pure E.coli solution



Experiment Name: 22/06/18
Specimen Name: 22/06/18
Tube Name: 2 NUMARA
Record Date: Jun 23, 2018 12:31:32 PM

Population	#Events	%Parent	%Total
BACTERIA	9,190	3.3	3.3
DEAD	1,516	16.5	0.5
LIVE	6,262	68.1	2.2
INJURED	1,273	13.9	0.5

Figure 4.8 Number of bacteria (live/dead/injured) in E.Coli saline solution after collecting the Non-treated iron oxide nanoparticles from the same solution (GroupA2)



Experiment Name: 22/06/18
Specimen Name: 22/06/18
Tube Name: 1 NUMARA
Record Date: Jun 23, 2018 12:22:54 PM

Population	#Events	%Parent	%Total
BACTERIA	8,649	8.0	8.0
DEAD	720	8.3	0.7
LIVE	7,467	86.3	6.9
INJURED	396	4.6	0.4

Figure 4.9 Number of bacteria (live/dead/injured) in E.Coli saline solution after collecting the biotin-coated iron oxide nanoparticles from the same solution (Group B2)

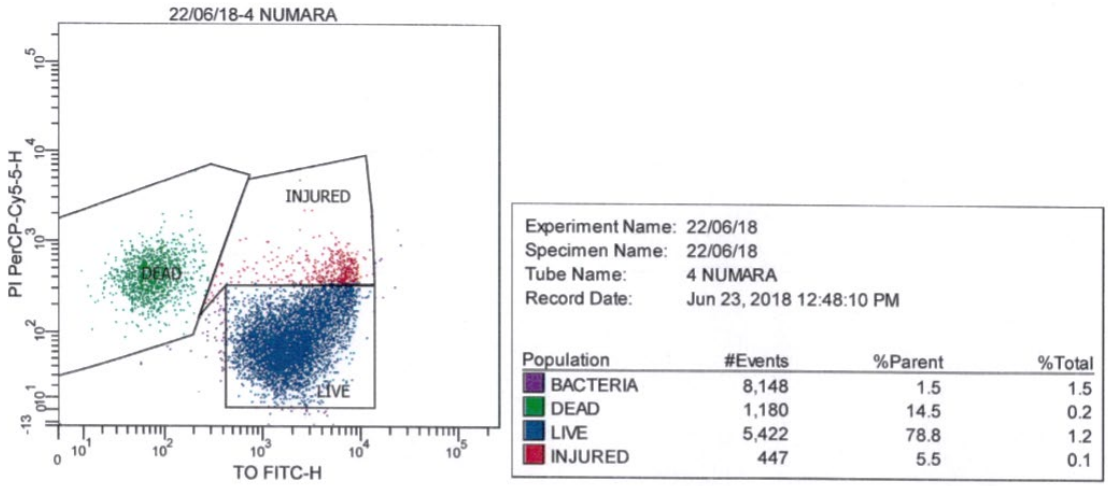


Figure 4.10 Number of bacteria (live/dead/injured) in E.Coli saline solution after collecting the streptavidin-coated iron oxide nanoparticles from the same solution (Group C2)

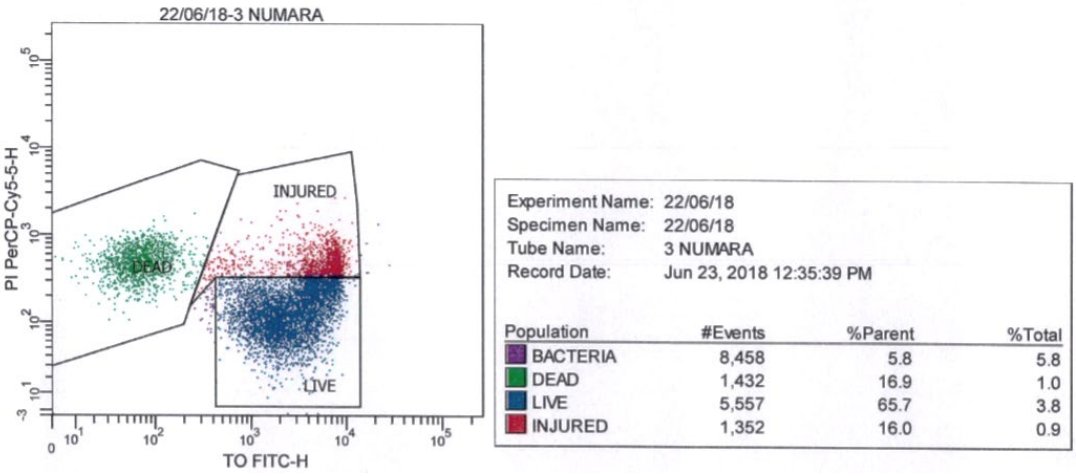


Figure 4.11 Number of bacteria (live/dead/injured) in E.Coli saline solution after collecting the streptavidin+biotin coated iron oxide nanoparticles from the same solution (Group D2)

Table 4.4 Effects of Volume of Bacteria-Mixed Saline on Collecting Number of Bacteria (live/dead/injured) in E.Coli saline solution after collecting the treated/nontreated iron oxide nanoparticles from E.coli saline solution

Group Names	Number of Bacteria	Number of Live Bacteria	Number of dead Bacteria	Number of injured Bacteria
Pure Ecoli Solution (Group 0)	10.000	9.249	519	147
Solutıon After E.coli Collected by Nanoparticles. (Group A2)	9.190	6.262	1.516	1.273
Solutıon After E.coli Collected by Nanoparticles Coated with Biotin (Group B2)	8.649	7.467	720	396
Solutıon After E.coli Collected by Nanoparticles Coated with Streptavidin (Group C2)	8.148	5.422	1.180	447
Solutıon After E.coli Collected by Nanoparticles Coated with Streptavidin and Biotin (Group D2)	8.458	5.557	1.432	1.352

By keeping the bacteria saline solution concentration constant, the amount of bacteria-mixed saline was increased to two times. It was observed that whether the success of nanoparticles in bacterial capture has increased with respect to the same solution without the increased volume of the bacteria solution.

The solution of 160 ml bacterial saline solution was placed into individual beakers and iron oxide nanoparticles were added (Group A2, Group B2, Group C2 and Group D2) to the solutions respectively. In order to collect these iron oxide nanoparticles, the magnetic field was applied.

Flow cytometry was used to count the live, dead and injured bacteria in the remaining bacterial saline after collection. When the measurements were taken into consideration, the amount of bacterial saline solution was increased from 80 ml to 160 ml and it was observed that 0.2 gram iron oxide nanoparticles were successfully collecting E.coli bacteria from the saline solution.

In addition, as stated in section 4.1, it was observed that when biotin was used alone, the ability to bind the iron oxide nanoparticles to E.coli bacteria was lower than streptavidin coated nanoparticles. It was found that the nanoparticles, which had not been treated in any way, had the ability to remove some amount of bacteria by carrying them with a little bit compared to the nanoparticles treated. Both the biotin and the streptavidin-binding iron oxide nanoparticles were not as successful as the streptavidin-bound iron oxide nanoparticles.

4.3. Effects of Aptamer Attached Nanoparticles to Collecting E.Coli Bacteria (Live/Dead/Injured) from E.Coli Saline Solution

Biotin and streptavidin were successfully attached to iron oxide nanoparticles in our previous studies in section 4.1 and 4.2. The aptamer was modified by Cerrahpaşa University (modification of aptamer in chapter 3). The main purpose of the studies in this part was to observe whether aptamer bonded iron oxide nanoparticles were more successful than iron oxide nanoparticles without aptamer. Iron oxide nanoparticles, which was 0.1 grams, were placed in individual beakers. Each group was named Group A3, Group B3, Group C3 and Group D3. Uncoated iron oxide nanoparticles were placed in Group A3. 0.1ml of streptavidin were poured onto the iron oxide nanoparticles in Group B3 and it was dried at 35° C.

In order to prepare iron oxide nanoparticles in Group C3, 0.05 ml streptavidin was added onto the nanoparticles and dried at room temperature, followed by addition of 0.05 ml of biotin and drying at room temperature. In order to prepare iron oxide nanoparticles in Group D3, 0.05 ml of streptavidin was added onto the nanoparticles and dried at room temperature, followed by addition of 0.05 ml of biotin and drying at room temperature. In another beaker, 0.05 ml of biotin was added to the modified aptamer and then this solution poured to nanoparticles in Group D3 and dried at 25 °C.

Table 4.5 Processed Nanoparticles

No	Group Name	Processed Nanoparticles
1	Group A3	Non-treated nanoparticles
2	Group B3	Streptavidin-coated and dried nanoparticles
3	Group C3	Streptavidin and Biotin coated and dried nanoparticles
4	Group D3	Streptavidin-biotin coated and attached aptamer nanoparticles

Bacterial isolates obtained from Cerrahpaşa University were dissolved in 400 ml of saline solution. The prepared 400 ml bacterial-saline solution was divided into five equal portions of 80 ml. 80 ml bacterial-saline solution poured into the four beakers individually, iron oxide nanoparticles were added into the beakers and named Group A3, Group B3, Group C3, Group D3. Iron oxide nanoparticles were collected from bacteria-mixed saline using a magnetic field. Each group was exposed to a magnetic field for 45 minutes.

Flow cytometry was used to count the live, dead and injured bacteria in the remaining bacterial saline solution after collection of the bacteria.

A kit was used to stain bacteria for flow cytometry measurement. In order to stain the bacteria in the solution, 500 µL of dye solution were used for each 5.0 µL of bacteria solution. Each dye solution contains 420 nmol/L for Tiozole Orange (TO) and 43 µmol/L for propidium iodide (PI).

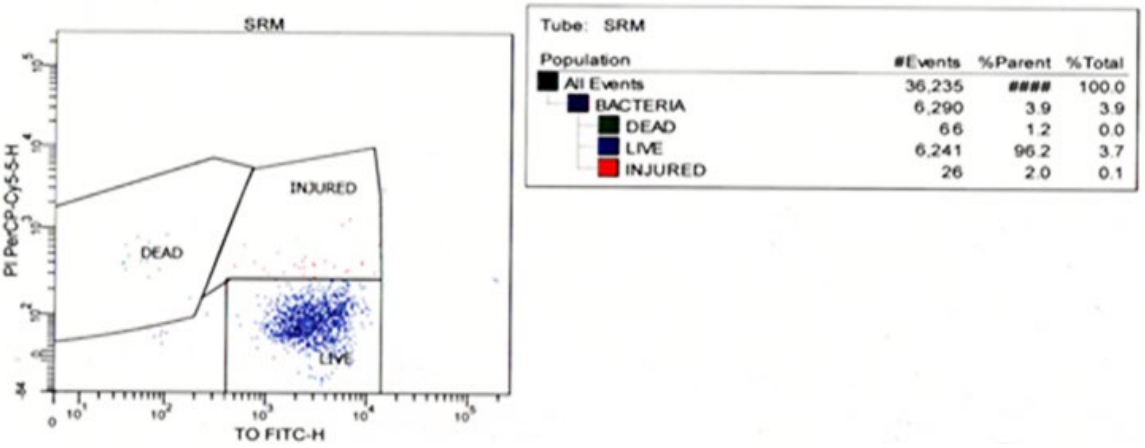


Figure 4.12 Flow cytometry measurements result for Pure E.coli solution

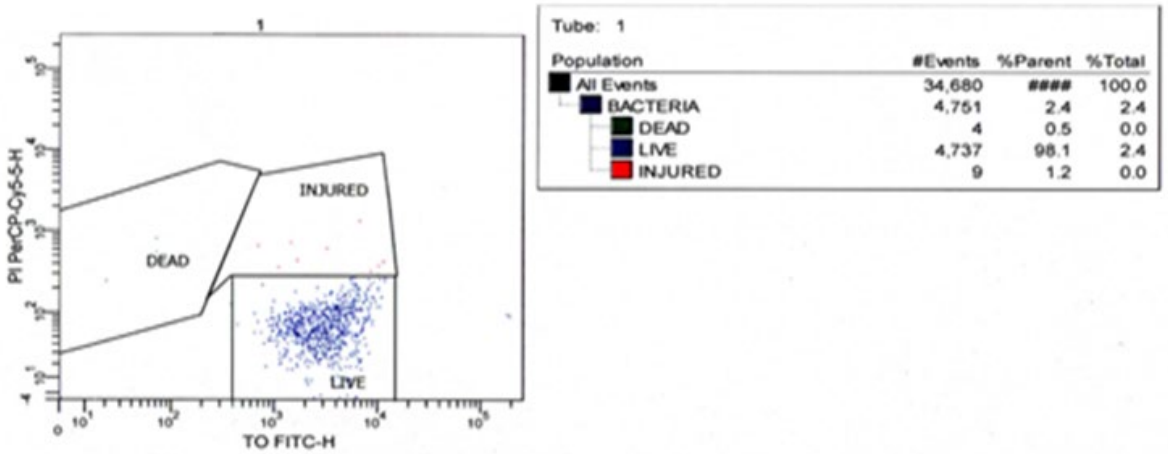


Figure 4.13 Number of bacteria (live/dead/injured) in E.Coli saline solution after collecting the untreated iron oxide nanoparticles from the same solution (Group A3)

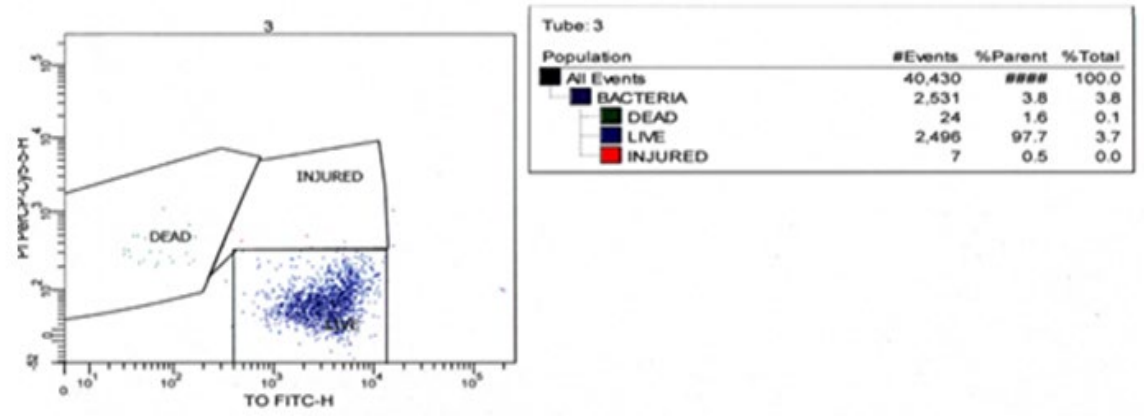


Figure 4.14 Number of bacteria (live/dead/injured) in E.Coli saline solution after collecting the streptavidin-coated iron oxide nanoparticles from the same solution (Group B3)

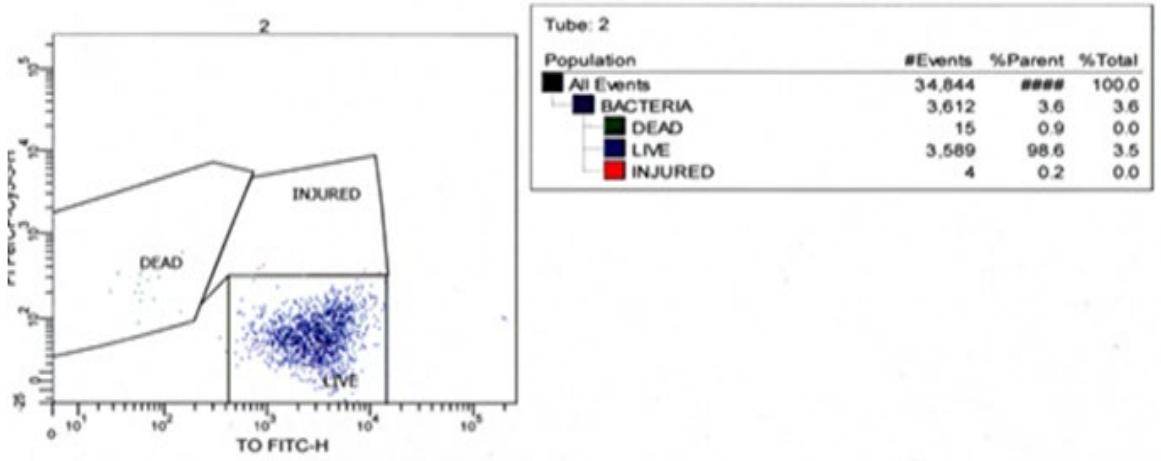


Figure 4.15 Number of bacteria (live/dead/injured) in E.Coli saline solution after collecting the streptavidin+biotin coated iron oxide nanoparticles from the same solution (Group C3)

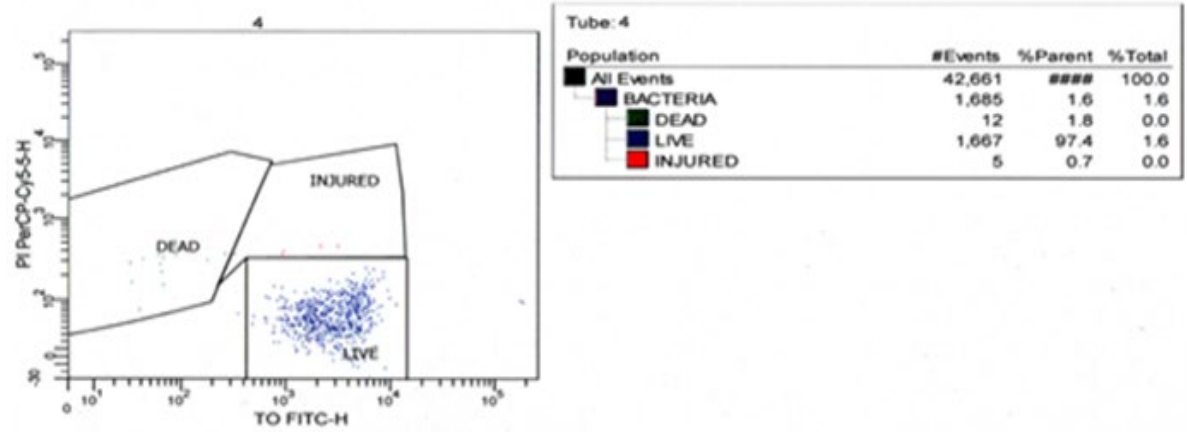


Figure 4.16 Number of bacteria (live/dead/injured) in E.Coli saline solution after collecting the streptavidin+biotin coated and attached aptamer iron oxide nanoparticles from the same solution (Group D3)

Table 4.6 Effects of Aptamer Attached Nanoparticle on Collecting E.coli Bacteria (Live/Dead/Injured) From E.Coli saline solution after collecting the treated/nontreated iron oxide nanoparticles from E.coli saline solution

Group Name	Number of Bacteria	Number of Live Bacteria	Number of Dead Bacteria	Number of injured Bacteria
Pure E.coli Solution **(Group 0)**	6.290	6.241	66	26
Solution After E.coli Collected by Nanoparticles**(Group A3)**	4.751	4.737	4	9
Solution After E.coli Collected by Nanoparticles Coated with Streptavidin **(Group B3)**	2.531	2.496	24	7
Solution After E.coli Collected by Nanoparticles Coated with Streptavidin And Biotin **(Group C3)**	3.612	3.589	15	4
Solution After E.coli Collected by Nanoparticles Coated with Streptavidin-Biotin and attached aptamer **(Group D3)**	1.685	1.667	12	5

Since the bacteria used for Aptamer DNA are synthesized specifically to the bacteria DNA, like a key lock fit between the bacteria and the aptamer is observed, which makes it easier to remove bacteria from the environment. In Chapter 4.3, iron oxide nanoparticles were attached to aptamer using streptavidin-biotin. It can be seen in table 4.5, among the bacterial solution samples, less bacteria remained in the sample which was aptamer attached nanoparticles were used to capture E. coli bacteria. In other words, more bacteria were collected in the bacterial solution sample which was aptamer attached nanoparticles were used to capture E. coli bacteria.

4.4. Effects of Volume of Water Sample When the Bacteria were Collected by Aptamer Attached Nanoparticles

It was observed in section 4.3, iron oxide nanoparticles functionalized by the aptamer are able to remove more bacteria from the bacteria solution with respect to the iron oxide nanoparticles with streptavidin/biotin/streptavidin+biotin coated nanoparticles. Based on the observations made in section 4.2 and 4.3, the amount of bacterial solution was doubled and the aptamer attached nanoparticles used to collect bacteria from E.coli saline solutions.

From 1 gram of iron oxide nanoparticles,two parts were taken and 0.2 grams each. Iron oxide nanoparticles, which was 0.2 grams, were placed in separate beakers. Each group was named Group A4, Group B4. 0.1 ml of streptavidin was added onto the iron oxide nanoparticles in Group A4 and dried at room temperature, followed by addition of 0.1 ml of biotin and drying at room temperature. 0.1 ml of streptavidin was added into the iron oxide nanoparticles in Group B4 and dried at room temperature, followed by addition of 0.1 ml of biotin and drying at room temperature. In another beaker, 0.1 ml of biotin was added to the modified aptamer from Cerrahpaşa University and added to the nanoparticles in Group B4 and dried at 25 ° C.

Table 4.7 Processed Nanoparticles

No	Group Name	Processed Nanoparticles
1	Group A4	Streptavidin and Biotin coated and dried nanoparticles
2	Group B4	Streptavidin-biotin coated and attached aptamer nanoparticles

Bacterial isolates obtained from Cerrahpaşa University were dissolved in 400 ml saline. From 400 ml bacteria-saline solution two parts of 160 ml was taken.

To the 160 ml bacterial-saline solution poured into the two beakers individually, iron oxide nanoparticles were added into the beakers and named Group A4, Group B4. Iron oxide nanoparticles were collected from bacteria-mixed saline using a magnetic field. Each group was exposed to a magnetic field for 45 minutes. In order to stain the bacteria in the solution, 500 µL of dye solution were used for each 5.0 µL of bacteria solution.Each dye solution contains 420 nmol/L for Tiozole Orange (TO) and 43 µmol/L for propidium iodide (PI).

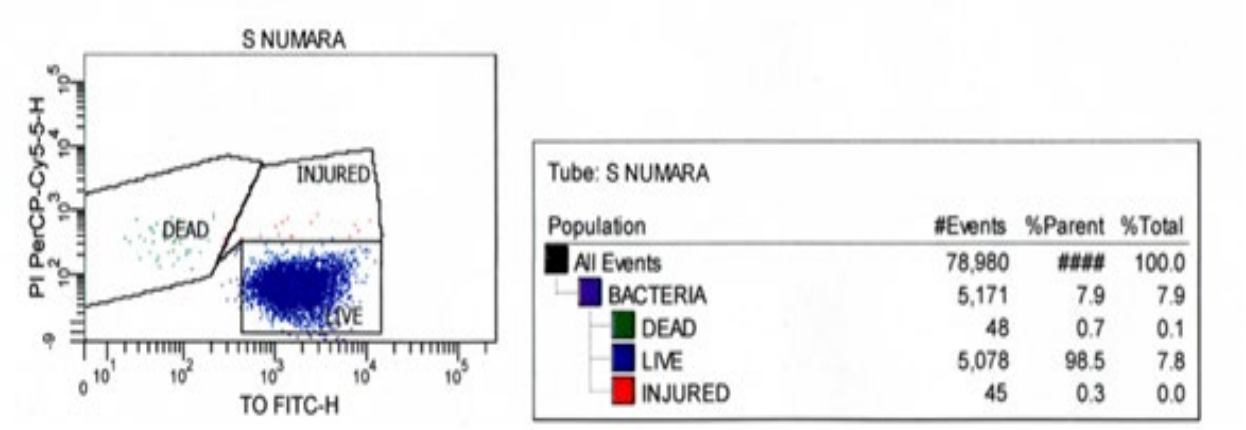


Figure 4.17 Flow cytometry measurements result for Pure E.coli solution

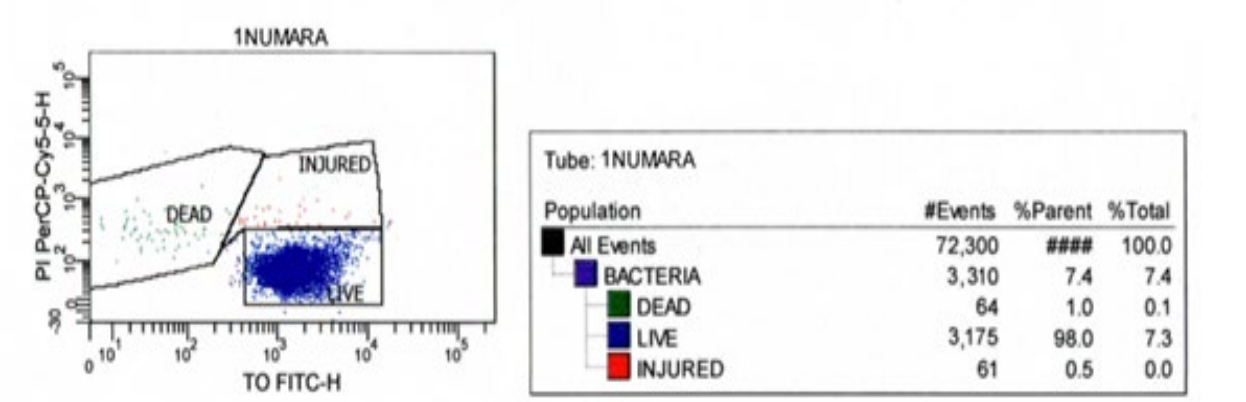


Figure 4.18 Number of bacteria (live/dead/injured) in E.Coli saline solution after collecting the streptavidin+biotin coated iron oxide nanoparticles from the same solution (Group A4)

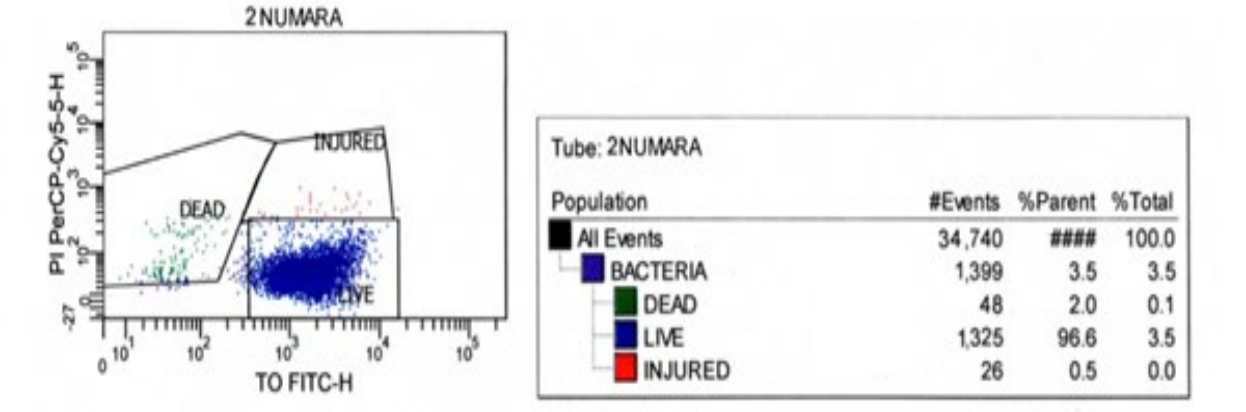


Figure 4.19 Number of bacteria (live/dead/injured) in E.Coli saline solution after collecting the streptavidin+biotin coated and attached aptamer iron oxide nanoparticles from the same solution (Group B4)

Table 4.8 Effects of Volume of Water Sample on Number of Bacteria (Live/Dead/Injured) When It is Collected by Aptamer Attached Nanoparticle after collecting the treated/nontreated iron oxide nanoparticles from E.coli saline solution

Process Groups	Number of Bacteria	Number of Live Bacteria	Number of Dead Bacteria	Number of injured Bacteria
Pure E.coli Solutıon **(Group 0)**	5.171	5.078	48	45
Solutıon After E.coli Collected by Nanoparticles Coated with Streptavidin-Biotin **(GroupA4)**	3.310	3.175	64	61
Solutıon After E.coli Collected by Nanoparticles Coated with Streptavidin-Biotin and attached aptamer **(GroupB4)**	1.399	1.325	48	26

In previous chapter 4.2 we find out that when the volume of the bacteria solution and amount of the nanoparticle was doubled it is more succesfull to collect E.coli bacteria from the solution. In chapter 4.3 table 4.5 shows that, among the bacterial solution samples, less bacteria remained in the sample which was aptamer attached nanoparticles were used to capture E. coli bacteria. In other words, more bacteria were collected in the bacterial solution sample which was aptamer attached nanoparticles were used to capture E. coli bacteria. In this study these two features were used together.

Flow cytometry was used to count the live, dead and injured bacteria in the remaining bacterial saline solution after collection of the bacteria. Iron oxide nanoparticles functionalized by the aptamer are able to remove more bacteria from the bacteria solution with respect to the iron oxide nanoparticles with streptavidin/biotin/satreptavidin+biotin coated nanoparticles.

It can be seen from Table 4.7 that since the bacteria used for Aptamer DNA are synthesized specifically to the DNA of the E.coli bacteria, like a key lock fit between the bacteria and the aptamer is observed, which makes it easier to remove bacteria from the environment.160 ml of bacteria-saline solution placed in individual beakers were poured on to the iron oxide nanoparticles labelled as GroupA4, GroupB4 and these iron oxide nanoparticles were collected by applying a magnetic field.We observe the aptamer attached iron oxide nanoparticles were more successful when the volume of the bacteria solution doubled.In GroupA4 there are more bacteria than GroupB4. When the flow cytometry measurements were taken into consideration, the amount of bacterial saline solution was increased from 80 ml to 160 ml and it was observed that 0.2 gram aptamer attached iron oxide nanoparticles were successfully collecting E.coli bacteria from the saline solution.

4.5. Effects of Timing in order to Attache Aptamer to Nanoparticle to Collecting Bacteria (Live/Dead/Injured) from E.coli Saline Solution

It was observed in section 4.3 and 4.4 that aptamer attached nanoparticles are successful to bind bacteria. It has been tried to determine the time required for the maximum number of aptamers to be bonded to the streptavidine and biotin coated nanoparticle. In addition, the effect of the refrigerator environment on the binding process of bacteria and aptamers was investigated in order to benefit from the results.

A total amount of 1 gram nanoparticle were synthesized. Iron oxide nanoparticles, which was 0.1 grams, were placed separately in eight beakers.

Firstly, 0.05 ml of streptavidin was added to the iron oxide nanoparticles and dried at room temperature, followed by addition of 0.05 ml of biotin and drying at room temperature. In another beaker, 0.05 ml of biotin was poured to the modified aptamer and mixed well. After that process, biotin aptamer solution was added to the nanoparticles and dried at 25 °C. Beakers were named Group A5, Group B5, Group C5, Group D5, Group E5, Group F5, Group G5, Group H5.

Table 4.9 Processed Nanoparticles

Group Name	**Processed Nanoparticles**
Group A5	0.5 hours held outside the refrigerator(collected with nonaptamer attached nanoparticle)
Group B5	0.5 hours held outside the refrigerator(collected with aptamer attached nanoparticles)
Group C5	0.5 hours held in the refrigerator (collected with nonaptamer attached nanoparticle)
Group D5	0.5 hours held in the refrigerator (collected with aptamer attached nanoparticles)
Group E5	1 hour held outside the refrigerator (collected with nonaptamer attached nanoparticle)
Group F5	1 hour held outside the refrigerator (collected with aptamer attached nanoparticles)
Group G5	4 hours held outside the refrigerator (collected with nonaptamer attached nanoparticle)
Group H5	4 hours held outside the refrigerator (collected with aptamer attached nanoparticles)

Bacterial isolates obtained from Cerrahpaşa University were dissolved in 700 ml of saline solution. The prepared 700 ml bacterial-saline solution was divided into eight equal portions of 80 ml. To the 80 ml bacterial-saline solution poured into the eight beakers individually, iron oxide nanoparticles were added into the beakers and named Group A5, Group B5, Group C5, Group D5, Group E5, Group F5, Group G5, Group H5. Iron oxide nanoparticles were collected from bacteria-mixed saline using a magnetic field. Each group was exposed to a magnetic field for 45 minutes. In order to stain the bacteria in the solution, 500 µL of dye solution were used for each 5.0 µL of bacteria solution. Each dye solution contains 420 nmol/L for Tiozole Orange (TO) and 43 µmol/L for propidium iodide (PI).

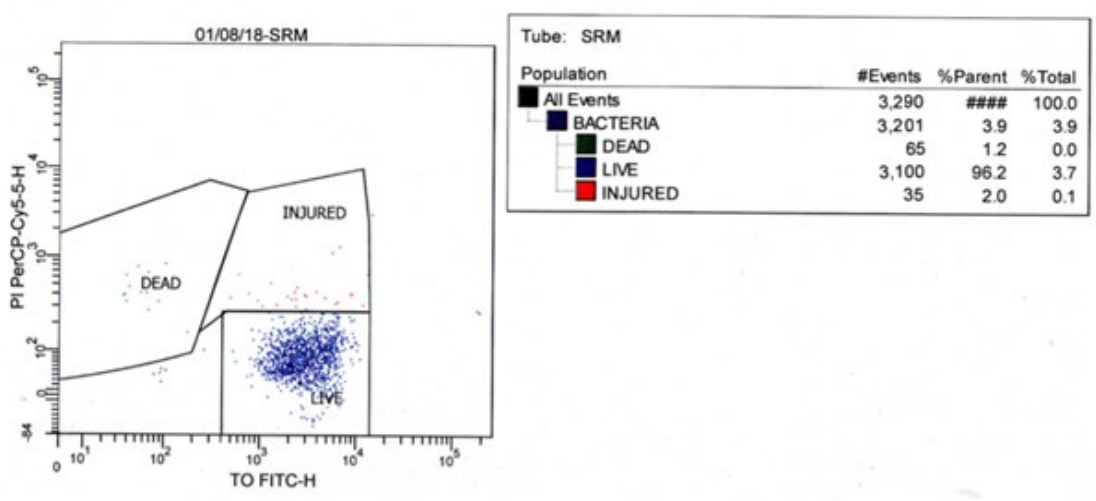


Figure 4.20 Flow cytometry measurements result for Pure E.coli solution

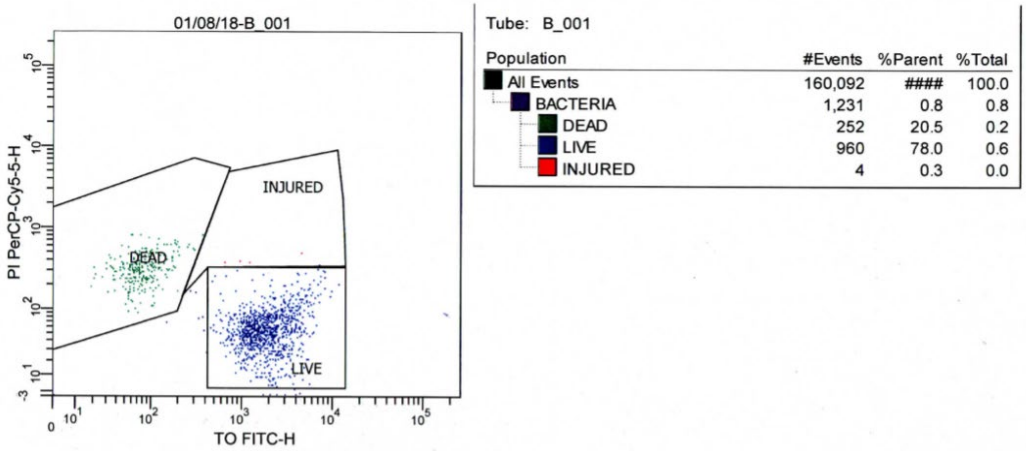


Figure 4.21 Number of bacteria (live/dead/injured) in E.Coli saline solution(which is 0.5 hours kept outside the refrigerator) after collecting the nonaptamer attached iron oxide nanoparticles from the same solution (GroupA5)

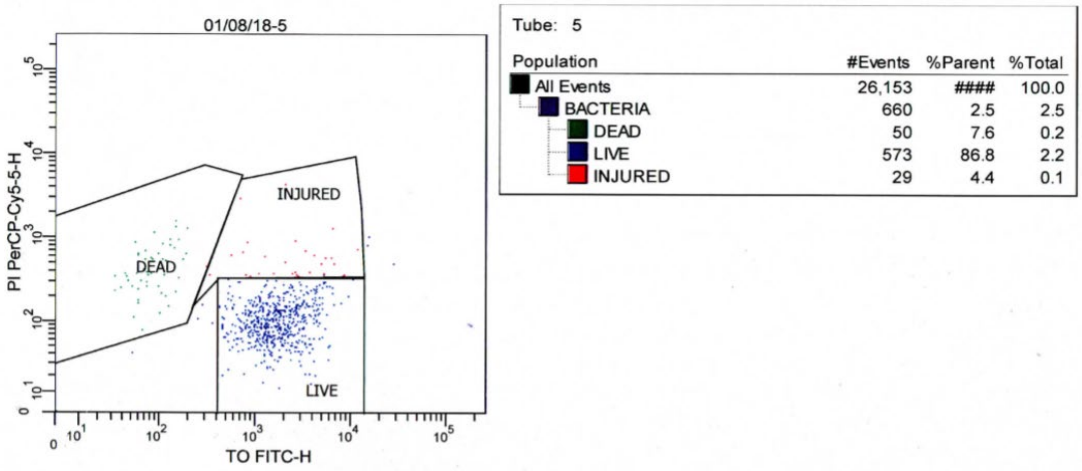


Figure 4.22 Number of bacteria (live/dead/injured) in E.Coli saline solution (which is 0.5 hours kept outside the refrigerator) after collecting the aptamer attached iron oxide nanoparticles from the same solution (GroupB5)

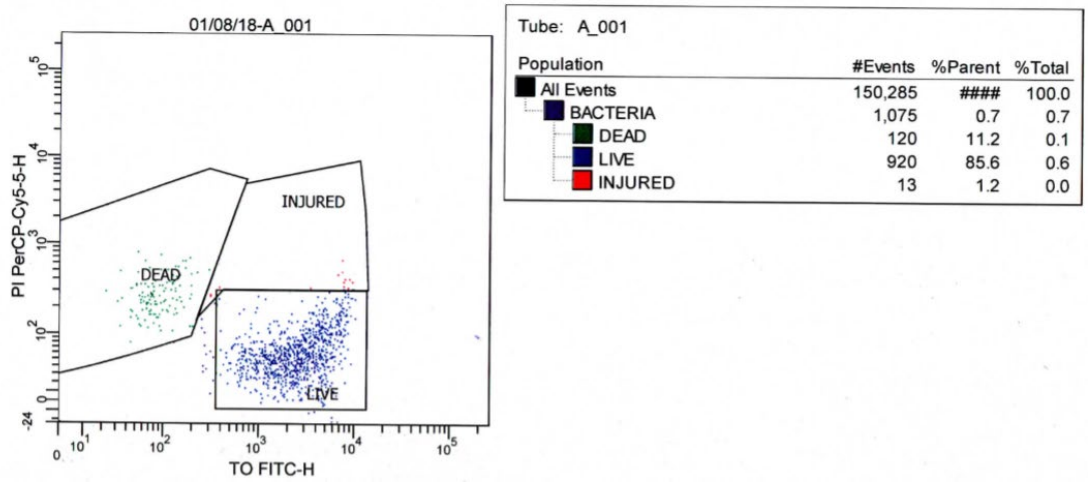


Figure 4.23 Number of bacteria (live/dead/injured) in E.Coli saline solution(which is 0.5 hours held in the refrigerator) after collecting the nonaptamer attached iron oxide nanoparticles from the same solution (Group C5)

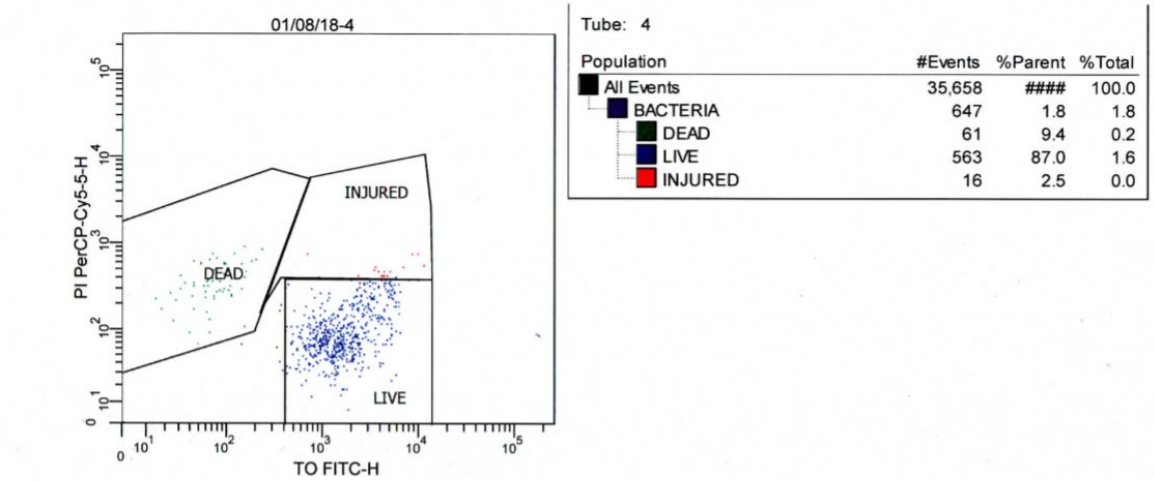


Figure 4.24 Number of bacteria (live/dead/injured) in E.Coli saline solution (which is 0.5 hours held in the refrigerator)after collecting the aptamer attached oxide nanoparticles from the same solution (Group D5)

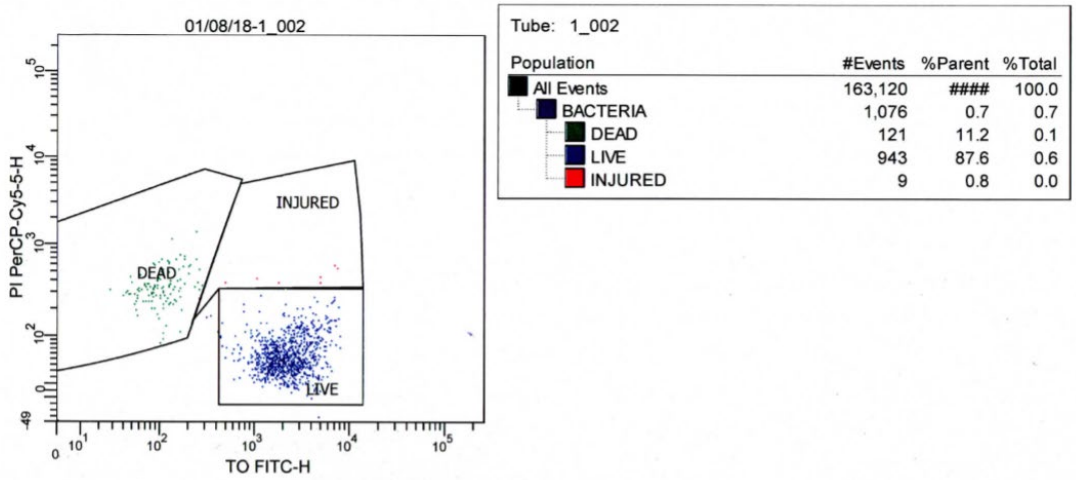


Figure 4.25 Number of bacteria (live/dead/injured) in E.Coli saline solution (which is 1 hour kept outside the refrigerator)after collecting the nonaptamer attached iron oxide nanoparticles from the same solution (Group E5)

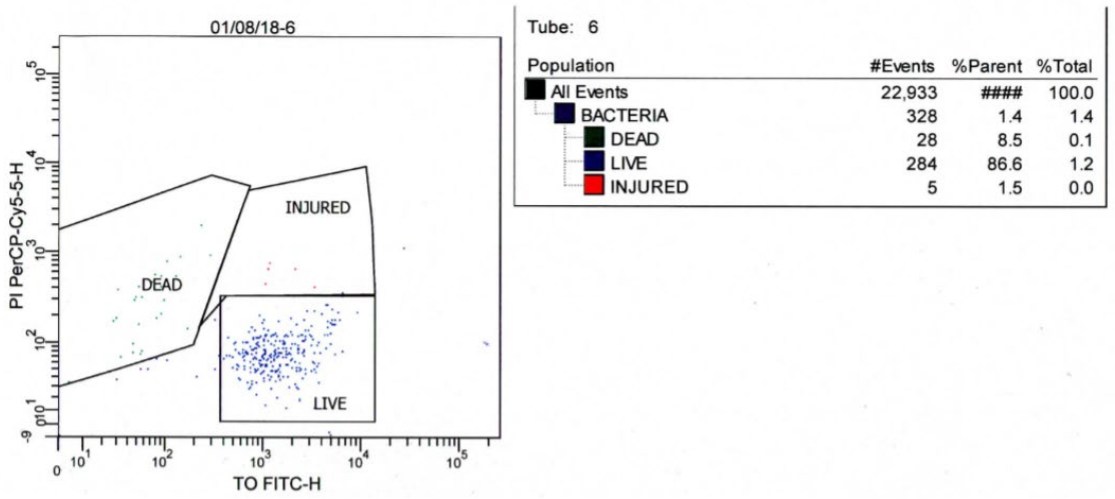


Figure 4.26 Number of bacteria (live/dead/injured) in E.Coli saline solution(which is 1 hour kept outside the refrigerator)after collecting the aptamer attached iron oxide nanoparticles from the same solution (Group F5)

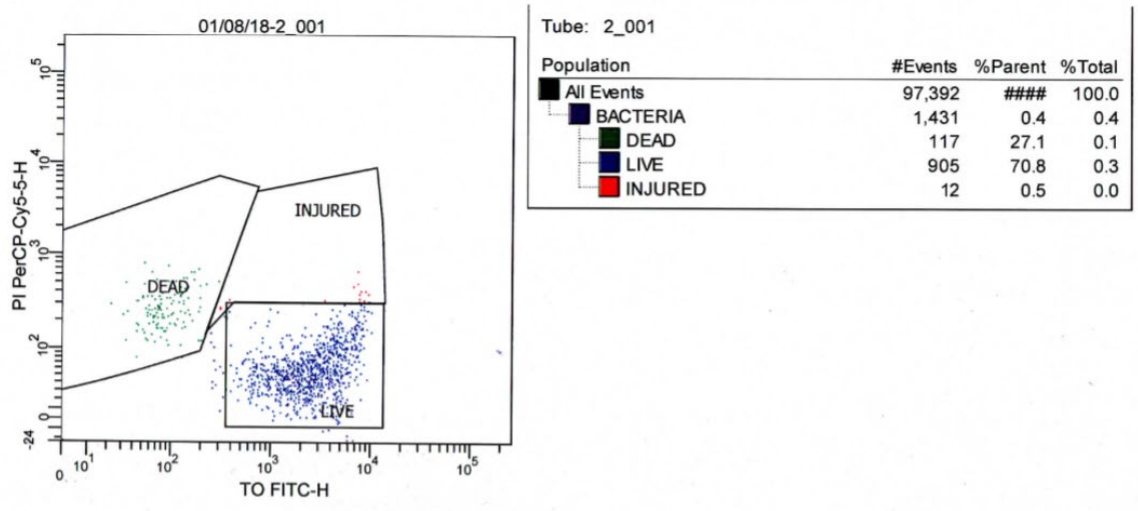


Figure 4.27 Number of bacteria (live/dead/injured) in E.Coli saline solution(which is 4 hours kept outside the refrigerator) after collecting the nonaptamer attached iron oxide nanoparticles from the same solution (Group G5)

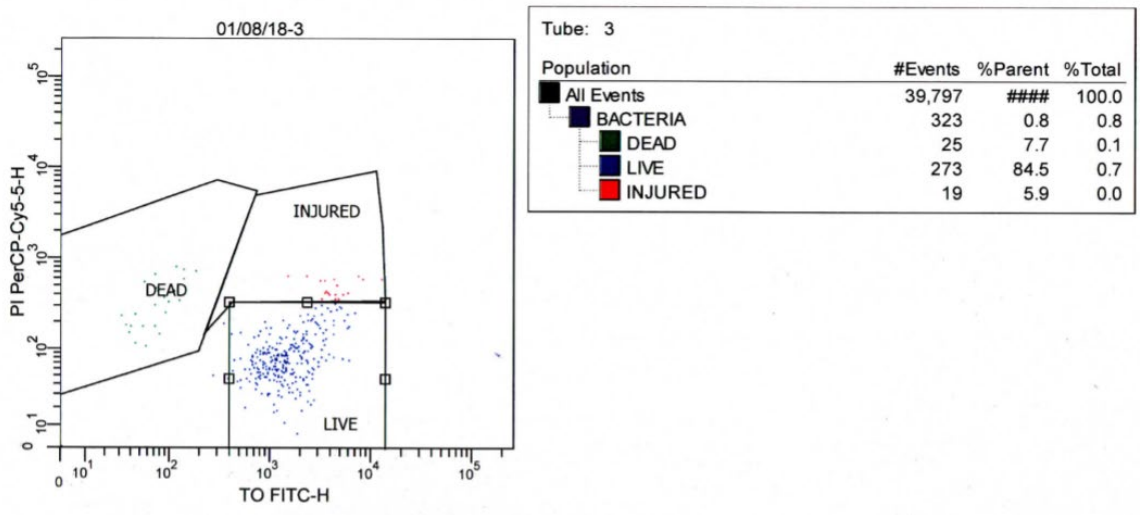


Figure 4.28 Number of bacteria (live/dead/injured) in E.Coli saline solution (which is 4 hours kept outside the refrigerator) after collecting the aptamer attached iron oxide nanoparticles from the same solution (Group H5)

Table 4.10 Effects of time interval in order to Attache Aptamer to Nanoparticle to Collecting Bacteria (Live/Dead/Injured) from Water Sample after collecting the treated/nontreated iron oxide nanoparticles from E.coli saline solution

Process Groups	**Number of Bacteria**	**Number of live Bacteria**	**Number of dead Bacteria**	**Number of injured Bacteria**
Group 0	3.201	3.100	65	35
Group A5	1.231	960	252	4
Group B5	660	573	50	29
Group C5	1.075	920	120	13
Group D5	647	563	61	16
Group E5	1.076	943	121	9
Group F5	328	284	28	5
Group G5	1.431	905	117	12
Group H5	323	273	25	19

Group 0 ; Flow cytometry measurements of pure e.coli solution

In this section, it has been tried to determine the time required for the maximum number of aptamers to be bonded to the streptavidine and biotin coated nanoparticle. For this purpose, it was thought that aptamer would have the optimum binding time to connect to the maximum number of streptavidine and biotin coated nanoparticle. In this study, the periods determined as 0,5 hours, 1 hour and 4 hours.

As a result, it was seen that the optimum time for the binding of aptamers to the streptavidine and biotin coated nanoparticle was determined as 1 hour. Less than one hour of applications to prevents to reach the maximum number of aptamers bounded to streptavidine and biotin coated nanoparticle. Our research showed that the refrigerator environment has no significant effect on the binding process of streptavidine and biotin coated nanoparticle to aptamers.

4.6. Effects of Amount of Aptamer in order to Attache Aptamer to Nanoparticle to Collect Bacteria (Live/Dead/Injured) from E.Coli Saline Solution

It was estimated that the increase in the amount of aptamer could lead to more bacteria binding to the iron oxide nanoparticles. For this purpose, a total of 1 gram of iron oxide nanoparticle was synthesized as described in the third chapter. Iron oxide nanoparticle groups of 0.1 grams each were placed in individual beakers. Each group was named GroupA6, GroupB6, GroupC6 and GroupD6.

For the iron oxide nanoparticles in Group A6, 1 ml of streptavidin was added and dried at room temperature, followed by addition of 1 ml of biotin and drying at room temperature. In another beaker, biotin was mixed with modified aptamer. 1ml of this mixture was added into the nanoparticles and dried at 25 ° C.

For the iron oxide nanoparticles in Group B6, 1 ml of streptavidin was added and then dried at room temperature, followed by addition of 1 ml of biotin and drying at room temperature. In another beaker, biotin was mixed with modified aptamer. 3ml of this mixture was added into the nanoparticles and dried at 25 ° C.

For the iron oxide nanoparticles in Group C6, 1 ml of streptavidin was added and dried at room temperature, followed by addition of 1 ml of biotin and drying at room temperature.
In another beaker, aptamer and biotin were mixed and added 6 ml from this mixture on to the nanoparticles and dried at 25 ° C.

For the iron oxide nanoparticles in Group D6, 1 ml of streptavidin was added and dried at room temperature, followed by addition of 1 ml of biotin and drying at room temperature.
In another beaker, aptamer and biotin were mixed and added 9 ml from this mixture on to the nanoparticles and dried at 25 ° C.

Table 4.11 Processed Nanoparticles and their group names

No	Group Name	Processed Nanoparticles
1	Group A6	Streptavidin and Biotin coated nanoparticles
2	Group B6	Streptavidin-biotin coated and attached aptamer on nanoparticles by using 3 ml of the biotin-aptamer mixture
3	Group C6	Streptavidin-biotin coated and attached aptamer on nanoparticles by using 6 ml of the biotin-aptamer mixture
4	Group D6	Streptavidin-biotin coated and attached aptamer on nanoparticles by using 9 ml of the biotin-aptamer mixture

Bacterial isolates obtained from Cerrahpaşa University were dissolved in 400 ml of saline solution. The prepared 400 ml bacterial-saline solution was divided into four equal portions of 80 ml. To the 80 ml bacterial-saline solution poured into the four beakers individually, iron oxide nanoparticles were added into the beakers and named Group A6, Group B6, Group C6, Group D6. Iron oxide nanoparticles were collected from bacteria-mixed saline using a magnetic field. Each group was exposed to a magnetic field for 45 minutes.

In order to stain the bacteria in the solution, 500 µL of dye solution were used for each 5.0 µL of bacteria solution. Each dye solution contains 420 nmol/L for Tiozole Orange (TO) and 43 µmol/L for propidium iodide (PI).

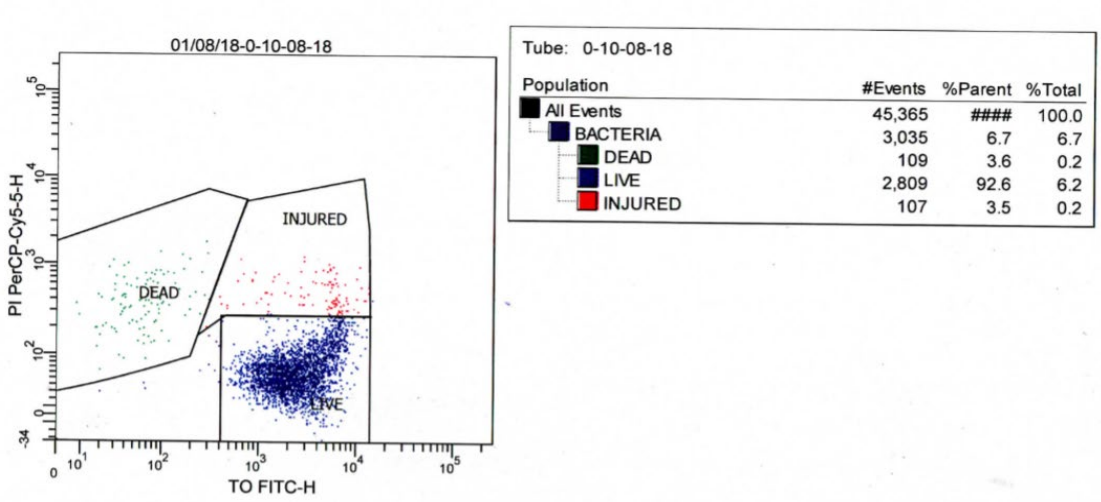


Figure 4.29 Flow cytometry measurements result for Pure E.coli solution

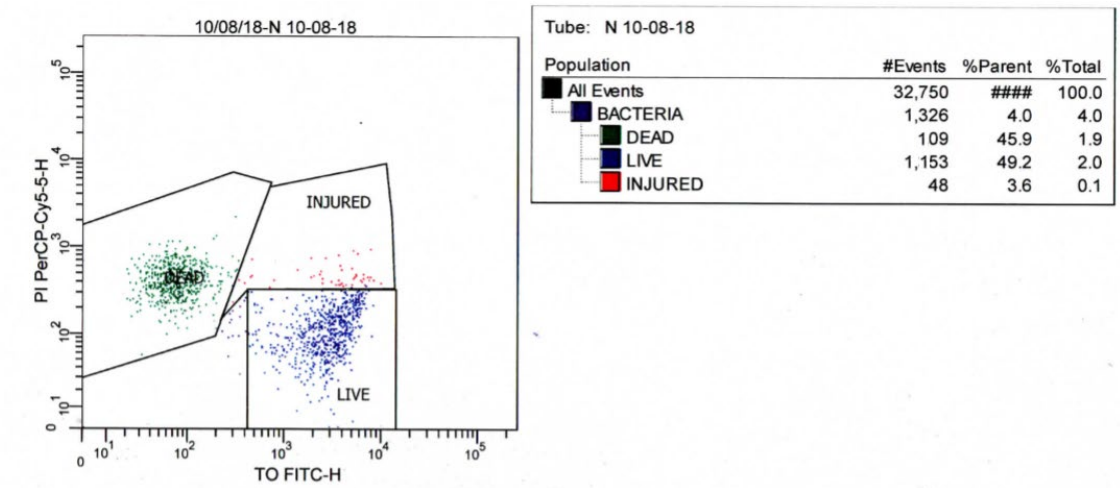


Figure 4.30 Number of bacteria (live/dead/injured) in E.Coli saline solution after collecting the streptavidin and biotin coated iron oxide nanoparticles from the same solution (Group A6)

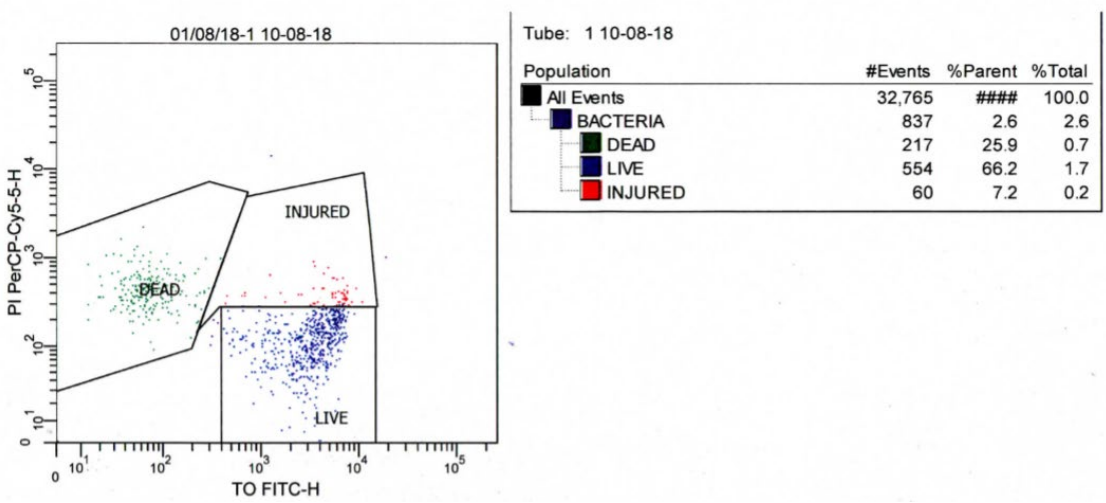


Figure 4.31 Number of bacteria (live/dead/injured) in E.Coli saline solution after collecting the aptamer (3 ml of the biotin-aptamer mixture) attached iron oxide nanoparticles from the same solution (Group B6)

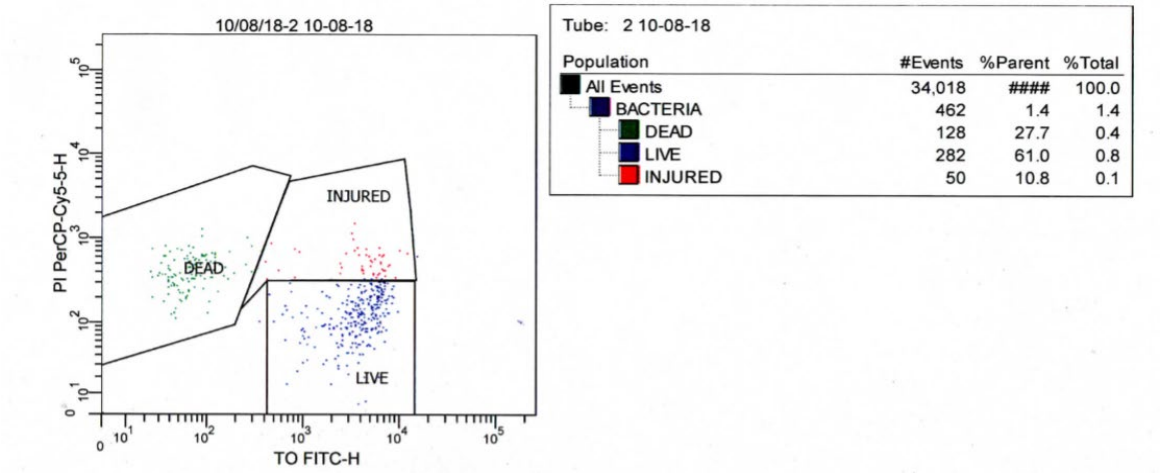


Figure 4.32 Number of bacteria (live/dead/injured) in E.Coli saline solution after collecting the aptamer (6 ml of the biotin-aptamer mixture) attached iron oxide nanoparticles from the same solution (Group C6)

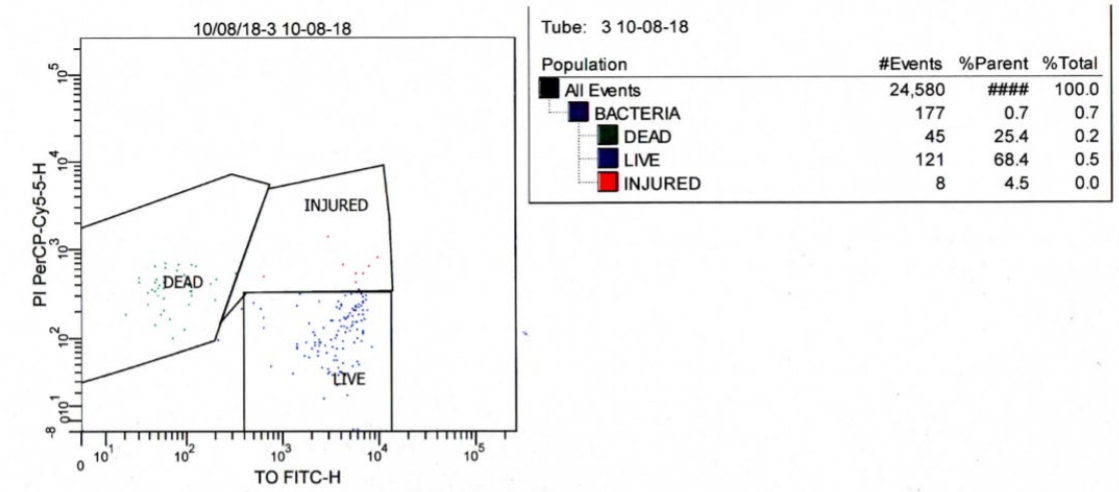


Figure 4.33 Number of bacteria (live/dead/injured) in E.Coli saline solution after collecting the aptamer (9 ml of the biotin-aptamer mixture) attached iron oxide nanoparticles from the same solution (Group D6)

Table 4.12 Effects Of Supply Proportion in order to Attache Aptamer to Nanoparticle to Collect Bacteria (Live/Dead/Injured) from E.Coli Saline Solution after collecting the treated/nontreated iron oxide nanoparticles from E.coli saline solution

Process groups	Number of Bacteria	Number of live Bacteria	Number of dead Bacteria	Number of injured Bacteria
Group 0	3.035	2.809	109	107
Group A6	1.326	1.153	109	48
Group B6	837	554	217	60
Group C6	462	282	128	50
Group D6	177	121	45	8

Group 0 : Flow cyctomerty measurements of pure E.coli solution

80 ml bacteria-saline solution placed in individual beakers were poured on to the iron oxide nanoparticles labelled as GroupA6, GroupB6, GroupC6 and GroupD6 and these iron oxide nanoparticles were collected by applying the magnetic field.

Flow cytometry was used to count the live, dead and injured bacteria in the remaining bacterial saline solution after collection of the bacteria.

It can be seen from Table 4.11, when the amount of aptamer-biotin mixture applied to the iron oxide nanoparticles increased, the number of bacteria bound to the nanoparticles increased too.

5. RESULTS AND DISCUSSION

In this study, a method has been proposed to demonstrate the availability of nanoparticles with magnetic properties (which can be controlled by externally applied magnetic fields), especially for the removal of E. coli from water sources. Iron oxide nanoparticles were synthesized, the surface of the nanoparticles was made functional with biotin, streptavidin, biotin+ streptavidin, biotin+streptavidine+aptamer. The effects of these modifications on magnetic nanoparticles on the ability to collect E-coli bacteria by binding them to iron oxide nanoparticles in order to reduction water samples from E.coli bacteria were investigated. The nanoparticles are efficient materials in terms of being light, compact, inexpensive, and more suitable for every application. It is been mentioned in the third section that iron oxide nanoparticles were prepared by using materials such as $FeCl_3.6H_2O$, D-Glucose($C_6H_{12}O_6$), Sodium hydroxide (NaOH) and distilled water with using the hydrothermal and high-temperature method. Our conclusions about results were given below in deteals for each parth (from 4.1 to 4.7) of the study.

In 4.1 effects of type of the binders on number of collecting bacteria (live/dead/ınjured) from the E.Coli saline solution was investigated. Variations in number of bacteria remaining/collected as persentece were presented below.

	Number of bacteria at initial	Percentage (%) of bacteria remaining in the solution after collecting nanoparticles	Percentage (%) of bacteria collected
Group 0	10 074	100	0
A1	10 031	99.5	0.5
B1	10 002	99.2	0.8
C1	9 178	91.1	8.9
D1	10 006	99.3	0.7

It has been observed that Streptavidin-coated on the biotin-coated iron oxide nanoparticles are not as successful as streptavidin coated iron oxide particles. By comparison the result for group B1 and D1 (Percentage (%) of bacteria collected 0.8% and 0.7% respectively), we conclude that streptavidin may not bound well onto the biotin layer by examining biotin coated iron oxide nanoparticles are not succesful to collect E.Coli bacteria. As seen from the table given above the most efficient method in order to collect E. Coli bacteria from the solution is using of streptavidin coated iron oxide particles.

In 4.2 effects of volume of bacteria-mixed saline on collecting number of bacteria (live/dead/ınjured) from e.coli saline solution was investigated. Variations in number of bacteria remaining/collected as percentage (%) were presented below.

	Number of bacteria at initial	Percentage (%) of bacteria remaining in the solution after collecting nanoparticles	Percentage (%) of bacteria collected
Group 0	10 000	100	0
A2	9 190	91.9	8.1
B2	8 649	86.4	13.6
C2	8 148	81.4	18,6
D2	8 458	84.5	15.5

By comparison the result for group 0 and A2(Percentage (%) of bacteria collected 0% and 8.1% respectively), it was found that the nanoparticles, which had not been treated in any way, had the ability to remove some amount of bacteria by carrying them with a little bit compared to the nanoparticles treated.It can be seen from the result for group B2 and D2(Percentage (%) of bacteria collected 13.6% and 15.5% respectively), Both the biotin and the streptavidin-binding iron oxide nanoparticles were not as successful as the streptavidin-bound iron oxide nanoparticles. By comparison the result for group C1 and C2 (Percentage (%) of bacteria collected 8.9% and 18.6% respectively), it was observed that whether the success of nanoparticles in bacterial capture has increased with respect to the same solution without the increased volume of the bacteria solution.In 4.1 and 4.2 the most succesfull results were obtained by streptavidine coted nanoparticles.

In 4.3 Effects of Aptamer Attached Nanoparticles to Collecting E.Coli Bacteria (Live/Dead/Injured) from E.Coli Saline Solution was investigated. Variations in number of bacteria remaining/collected as percentage (%) were presented below.

	Number of bacteria at initial	Percentage (%) of bacteria remaining in the solution after collecting nanoparticles	Percentage (%) of bacteria collected
Group 0	6 290	100	0
A3	4 751	75.5	24.5
B3	2 531	40.2	59.8
C3	3 612	57.4	42.6
D3	1 685	26.7	73.3

In Chapter 4.3, iron oxide nanoparticles were attached to aptamer using streptavidin-biotin. By comparison the result given the table above most succesfull results were obtained from aptamer containing nanoparticles.

By comparing with the privious results given the tables above aptamer is more efficent than streptavidine containing nanoparticles.

As mentioned in the second chapter, since the bacteria used for Aptamer DNA are synthesized specifically to the bacteria DNA, like a key lock fit between the bacteria and the aptamer is observed, which makes it easier to remove bacteria from the environment.

In 4.4 Effects of Volume of Water Sample When the Bacteria were Collected by Aptamer Attached Nanoparticles was investigated. Variations in number of bacteria remaining/collected as percentage (%) were presented below.

	Number of bacteria at initial	Percentage (%) of bacteria remaining in the solution after collecting nanoparticles	Percentage (%) of bacteria collected
Group 0	5 171	100	0
A4	3 310	64	36
B4	1 399	27	73

We observe the aptamer attached iron oxide nanoparticles were more successful to collect the bacteria when the volume of the bacteria solution is doubled. By comparison the result for group A4 and B4 (Percentage (%) of bacteria collected 36% and 73% respectively), it can be seen that in GroupA4 there are more bacteria than GroupB4.

In 4.5 Effects of Timing in order to Attache Aptamer to Nanoparticle to Collecting Bacteria (Live/Dead/Injured) from E.coli Saline Solution was investigated. Variations in number of bacteria remaining/collected as percentage (%) were presented below.

	Number of bacteria at initial	Percentage (%) of bacteria remaining in the solution after collecting nanoparticles	Percentage (%) of bacteria collected
Group 0	3 201	100	0
A5	1 231	38.4	61,6
B5	660	20.6	79,4
C5	1 075	33.5	66,5
D5	647	20.2	79.8
E5	1 076	33.6	66,4
F5	328	10.2	89.8
G5	1 431	44.7	55,3
H5	323	10	90

In this section, it has been tried to determine the time required for the maximum number of aptamers to be bonded to the streptavidine and biotin coated nanoparticle . For this purpose, it was thought that aptamer would have the optimum binding time to connect to the maximum number of streptavidine and biotin coated nanoparticle. In this study, the periods determined as 0,5 hour, 1 hour and 4 hours. By comparison the result for group F5 and B5(Percentage (%) of bacteria collected 89.8% and 79.4% respectively), it was seen that the optimum time for the binding of aptamers to the streptavidine and biotin coated nanoparticle was determined as 1 hour.

Less than one hour applications prevents to reach the maximum number of aptamers bounded to streptavidine and biotin coated nanoparticle. By comparin A5, C5 and B5,D5 there is no remarkeble effect of refrigerator on number of bacteria collected additionaly nanoparticles containing aptamer presented more efficency on collecting the number of bacteria as indicates of the previous study expressed above.

By compairing group B5, F5, H5 we can conclude that binding time interval for aptamer to

bound bacteria has possitive effects in order to collect the number of bacteria from the solution. It seems one hour is Optimum binding time interval for aptamer binding to streptavidine and biotin coated nanoparticle.

In 4.6 Effects of Amount of Aptamer in order to Attache Aptamer to Nanoparticle to Collect Bacteria (Live/Dead/Injured) from E.Coli Saline Solution was investigated. Variations in number of bacteria remaining/collected as percentage (%) were presented below.

	Number of bacteria at initial	Percentage (%) of bacteria remaining in the solution after collecting nanoparticles	Percentage (%) of bacteria collected
Group 0	3035	100	0
A6	1326	43.6	56.4
B6	837	27.5	72.5
C6	462	15.2	84.8
D6	177	5.8	94,2

Bacteria-saline solution placed in individual beakers and 3ml, 6ml, 9ml of the biotin-aptamer mixture were poured on to the iron oxide nanoparticles labelled as GroupA6, GroupB6, GroupC6 and GroupD6 and these iron oxide nanoparticles were collected by applying the magnetic field.

It is clear from the results (Percentage (%) of bacteria collected 72.5% and 84.8%,94.2% respectively), given in the table the more aptamer the more number of collected bacteria .
It was estimated in section 4.6 that the increase in the amount of aptamer could lead to more bacteria binding to the iron oxide nanoparticles.

Electrochemical DNA detection methods as a sign of nanoparticles or nanocrystals have become widespread in recent years and have been used in many different ways.

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CURRICULUM VITAE

PERSONAL INFORMATION
Surname, Name : TOPBAŞ TEKİN, Merve Pınar
Date and Place of Birth : 21.02.1991, İSTANBUL
Yabancı Dili : İngilizce

EDUCATION

Derece	Alan	Okul/Üniversite	Mezuniyet Yılı
Lisans	Fizik	Kocaeli Üniversitesi	2014

SERTIFICATE
Karşılaştırmalı C++ ve Fortran 90 yazılım sertifikası
İstanbul Üniversitesi, 2016
Araştırma Odaklı Monte Carlo Simülasyonu Uygulamaları Sertifikası
İstanbul Üniversitesi, 2016
Pedagojik Formasyon Sertifikası
Kocaeli Üniversitesi, 2014