Optimizing Colorimetric method for Laboratory Diagnosis of Bacillus Cereus Based Gold Oligonucleotide Nano Probes

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ABSTRACT

Introduction; Bacillus cereus is a Gram-positive, spore-forming bacterium which has known a major cause of food poisoning in the industrialized world. Many strains of the bacteria causing food poisoning like diarrhea and vomiting. Diarrheal diseases are created by hemolysin enterotoxins HBL, NHE and Non-hemolytic cytotoxin K. Bacillus cereus can be found mainly in milk. Bacillus cereus spores could well tolerate milk high temperature [boiling] and when exposed to room temperature germinates and grows. Purpose: The aim of this study was to optimize a rapid diagnostic method with highly sensitive. Methods: In order to optimizing nhe C gene was utilize which is one of B. cereus virulence gene and when exposed to room temperature germinates and grows. Conclusion: Bacillus cereus was detected and its detection was not visible to the eye. And obtained results were confirmed by infrared spectroscopy and spectrophotometry and TEM.

INTRODUCTION

Bacterial pathogens are a threat to human health. For this reason, identification of pathogens in food is important. [1, 2, 3, 4]. Among food pathogens, B. cereus is important food pathogens. [1, 5]. The bacteria can cause food poisoning. The bacterium is cause of food poisoning. And contaminating foods are including meat and meat products, vegetables, fish and seafood, milk, dairy products and prepared frozen foods. [4, 5]. Bacillus genus is from Bacillaceae families which has gram-positive optional aerobic non aerobic bacteria and have the ability to produce endospore. Only one species of this genus, Bacillus anthracis strains cause disease in humans and animals. There are other species of this genus such as B.subtilis and Bacillus licheniformis that have been identified as potential risk factors in dietary toxins. The distinguishing characteristic of members of this genus is their ability to survive in unfavorable conditions. The bad bacteria in the bad food condition can be converted into spores. [2]. There is extreme heterogeneity between organisms of the bacillus genus and also there is diversity in the types of metabolic reactions, nutritional requirements and the content of cell wall structure a vast difference between guanine and cytosine in DNA molecule. In this genus there are different kind of Psychrophiles, thermophile, mesophile and similarly Basophile and Neutrophil and Acidophile species:

Some members of the bacillus genus are antibiotics producer such as polymyxin and bacitracin which are clinically-useful. Other species of this genus as used in the production of industrial alcohol, enzymes and vitamins are important. [6]

Bacillus cereus is Gram-positive, facultative anaerobes and aerobes from Bacillaceae family and has flagella produce spores, usually motile, Haemolytic activity, Resistant to penicillin, catalase positive, negative mannotol, which contains phospholipase activation system.
This bacterium is widely distributed in nature. Among these bacteria, 2 bacteria tolerate coldness called Bacillus pseudomyoides and Bacillus vein stephen cis and recently a new pathogen named bacillus neocerus also based on gene sequencing studies and the percentage of G + C are in this group.[7].

Some Bacillus cereus strains, such as hemolysin producer strains are harmful but some of these strains are useful as probiotics for animals, [8, 9]

Bacillus cereus is moving in culture and that is why it is different with the other species in the distribution form in semisolid medium. [10]

Root growth of Bacillus cereus is not like Bacillus mycoides in the agar medium.

Bacillus cereus is mostly related to Bacillus anthracis, causes anthracis and Bacillus thuringiensis insect pathogens. But with differences in colony color, motility, hemolysin, Sensitivity to penicillin, Paraasprval objects, virulence factor in mice.

Bacillus cereus is commonly found in Trypticase soy in plate containing strong beta hemolytic sheep blood. While the Bacillus thuringiensis and Bacillus cereus mycoides are often poorly hemolytic. [11]

Bacillus anthracis is not moving and is not hemolytic therefore with this characteristic we can differentiate B. cereus from other Bacillus. [6, 11]

In recent years, increasing evidence of the role of Bacillus cereus in dietary toxins is presented. [12]

According to survey conducted in Finland in 2005, about 55 cases of food poisoning occurs when their reasons were food and water, 19 cases out of 55 cases, which means 38% reason remained unknown. In epidemic studies it has been identified that Ten percent of cases of food poisoning was due to food contaminated with these bacteria. [13]

Based on the survey conducted in 2006 found that Bacillus cereus is a fundamental problem in Europe is contaminated foods. [3, 14]

A number of Gram-positive bacteria under unfavorable environmental conditions become spores to escape the unfavorable environmental conditions.

These bacteria are resistant to UV and gamma rays, anti-bacterial elements, heat, drought, frost, chemical pesticides and other harmful environmental factors. [15]

Although the spores are resistant to unfavorable environmental conditions but they are inactive. The spores do not have multiply in the municipality. [6, 16]

Bacillus cereus spores may remain alive even during the heating process, so the germination of spores of Bacillus species is important to the study. [17]

Germination of spores of Bacillus cereus, at temperatures less than 15 °C occurs in rice more than soy so the strains of diarrheal are widespread more than Vomiting strains. [18]

Bacteria are being Phagocytes. Capsules are often during in vitro culture disappear. B. cereus produces a carbohydrate capsule. [19]

The main feature of the cell wall of Bacillus cereus is repetitive units of Tetra saccharide. [20]

The physical properties of the membrane during spore germination are changed and make a change in the lipid composition of the membrane. [12]

Growth and survival of Bacillus cereus depends on temperature, pH, oxygen and water activity. [22]

Bacillus cereus inhibiting factors are including; Nisin, sorbic acid, antibiotics such as: Aureomycin, Dihydrostreptomycin, Tobramycin, bacitracin, oxytetracycline, chloramphenicol and gentamicin and a slight inhibition by neomycin, Cloxacillin, ampicillin and penicillin was observed.

Clinical isolates of Bacillus cereus are the most susceptible to Imipenem, vancomycin and ciprofloxacin and rarely sensitive to Penicillin or cephalosporin except Mezlocillin. [23]

Bacillus cereus addition to causing gastrointestinal and intestinal disease by spores or toxins, are also capable of causing mastitis, systemic infection, Qarnarya, Mingingitisin children with immune deficiencies caused by drugs that inhibit the immune system, or malnutrition, respiratory infections, and other clinical problems [non-GI intestinal] are.[9]

A number of Bacillus cereus strains can be transmitted through food or cause food poisoning in the different types of foods. In 48 samples of food products such as raw milk, cheese and ice cream in order the amount of 14%, 35%, 9% of B. cereus was isolated. Flavored milk contains higher levels of Bacillus cereus. [24]

Bacillus cereus has been found in spring and autumn samples of fatty milk in amount of 4/71and 3/33%. During the pasteurization organisms remain stable in the milk. [24]

In Argentina, 433 honey samples were collected that 27 percent of Bacillus cereus from these samples and 14% from other Bacillus species were isolated. [25]

Phospholipase produced by Bacillus cereus. Phospholipase activity can be determined through observing turbidity zones on agar plates containing 1% egg yolk. 83 out of 94 isolated Bacillus cereus are belonging to Bacillus cereus species. 53% out of 83% isolated Bacillus cereus are producing NHE, HBL, Enterotoxin and Cytokinin K which typically are discussed as etiologic agents of Bacillus cereus diarrheal type food poisoning. One other isolated Bacillus is mycoides which are containing nheC and nheA genes. In Netherlands 3 enterotoxin coding genes are including: Cyto toxins k, NHE, HBL specific toxin produced vomiting [Cerolid] were studied and
among analyzed and examined genes NHE gene more than 97 percent and HBL approximately 66% and cytokine K gene approximately 50% were isolated and separated.[26]

When gold is produced at the Nano scale, the physical and chemical characteristics are undergoing many changes. Gold Nanoparticles maintain its activity at low temperatures

The advantage of this as catalysts in chemical reactions and pollution control are used.

In addition to the use of Gold Nanoparticles in drug and gene delivery in cancer treatment and they have been frequently developed for detection of microorganisms as biological sensors. [27, 28, 29, 30, 31].

**Purpose:**
These bacteria has a lot of polluters’ potential, but has a little virulent power and people with immune deficiency and cardiovascular disease, diabetes, alcoholism and with transplant as well as pregnant women, the elderly and infants are susceptible to infection by this bacterium.[1,5]

This bacterium is the cause of septicemia, meningitis, conjunctivitis, abortion and purulent infections in humans. In Infants is as a third leading cause of encephalitis after E-Coli and streptococcus agalactia. Due to these reasons, a method that makes it possible to detect the pathogen at low levels is extremely necessary.[2, 6]

Considering the low infectious dose of food pathogen, the presence of this pathogen at every stage of production, processing, distribution and storage of food products should be investigated. [1, 5]

So, the access to a sensitive diagnostic method, rapid, specific and operational capabilities to ensure the safety of food supplies is essential. [4, 1, 2, 3,]

Objectives to be pursued in this thesis include Optimizing Colorimetric method based on Gold oligonucleotide Nano probes for the diagnosis of Bacillus cereus and developing a highly sensitive method for the laboratory diagnosis of Bacillus cereus.

**Methods:**
In order to investigate the above-mentioned objectives, first Bacillus cereus DNA through Gram-positive bacteria extraction kits was extracted. In order to ensure proper extraction, extracted DNA was analyzed qualitatively and quantitatively. The extracted DNA was used for polymerase chain reaction test.

To perform the PCR test PCR gradient system was used to create various temperatures and finding the optimum temperature for gene amplification. The PCR product was also evaluated qualitatively and quantitatively. Then in order to perform colorimetric Gold oligonucleotide Nano probes were prepared and with nheC gene product of PCR was set. The sensitivity of this method is very high and provides a probability for detection in short time and requires no sophisticated equipment. In this study, through using a rapid and sensitive colorimetric method, Bacillus cereus species due its virulent importance than other species were identified. First a mixture of PCR product and Nano probe was under the denaturation temperature of 70 ° C. Then the temperature was gradually lowered and finally, the temperature was reached to 45 ° C and remained at this temperature for 30 min till connections between Nano probes and PCR product are done.

For this purpose, first based on the desired gene two probes are designed, now at the end of 3’ the probes and at the end of 5’ other probe a thiol group is added. Then the thiolated probes from the thiol end are connected to gold nanoparticles and desired Nano probes are made. Now based on gene PCR nheC is performed then the generated PCR product to examine the presence of nheC gene in specific binding temperature is added. At this temperature Nano probes are attached in specific part of desired gene and as the result of binding and accumulation our desired Solution color is change from red to purple. But if the gene is not available, no connection is made. Nano probes remain free in solution and solution color remains the same red color.

Lyoiphilised Bacillus cereus strains ATCC1154 which had confirmed bacteria test was taken from Department of Microbiology, Shahid Beheshti University of Medical Sciences and sub cultured in Mueller hinton agar medium. After this period, along the flame plates are examined and in the absence of colonies, were kept in the refrigerator for a week are useable.

In order to extract Bacillus cereus genome kit for extraction gram-positive was used. For this purpose, bacteria were inoculated into nutrient broth medium that had been incubated for 24 hours and Gram staining was used to ensure that there is no contamination. After precipitation and isolation of DNA, It was taken on an agarose gel and electrophoresis carried out to realize quality and quantity and its amount due to the sharpness and intensity of band. [32] Electrophoresis specific buffer or TBE 1X is provided stock at a concentration of 10x and while used it is diluted. [33] Primers, short pieces of single chain DNA length of 15-30 nucleotides that has been synthesized for a molecule with specific nucleotide sequences. The forward and reverse primers commonly used in the PCR that each of them have different sequences. So that Sequences of primers complementary to sequences that are located in the template DNA chains. PCR method is done through thermostable DNA polymerase. [34] Gene Runner software is used in this research to design primer for nheC gene. The primers duplicate region of 269 bp of nheC gene. Taq DNA polymerase is obtained from thermus aquaticus. Taq polymerase should be the last item to be added to the solution. Immediately the DNA polymerase starts its activities. Also Taq polymerase should be kept in the freezer until the last minute addition of a solution. After
adding the solution into a special machine put the polymerase chain reaction called PCR gradient to amplify of the desired gene. After polymerase chain reaction products were examined on agarose gel. After putting gel in electrophoresis Tank electrophoresis buffer should be at least 1 cm above the gel.

In order to load the sample into the lane, first the sample is mixed with loading buffer properly and with 5 to 1 Dilution and then fertilize into the lane.

First lane is DNA marker containing DNA fragments with different sizes and is used to identify the size of the possible examined fragment. After loading the samples, the electricity is connected and regulated on 80 volts.

After making the flow when the bromo phenol blue dye reached the gel bottom of a centimeter, electricity was cut off and cast out from the electrophoresis tank and to observe the Banding of DNA amplification gel documentation system used. Two oligonucleotide probes in domestic situations, complementary to PRC product were designed by Oligo5 software.

The specificity of the oligonucleotide sequences to identify target organism genes were analyzed using Blast online software. Since the probes are used, at the end of the 3' and 5' have thiol group, which includes the thiol-containing groups, is -SH.

This thiol group is oxidized to sustain probes and requires that before Conjugation reaction are reduced by a substance called DTT [Dithiothreitol]. NAP-5 columns are used to remove DTT.[35]

To delete non conjugated oligonucleotide, they were centrifuged four times with 13000 rpm for 25 min that a washing buffer containing Phosphate buffer 10 mM, sodium chloride 1.0 M and toin 80 was used. At this stage, excess DNA was washed out.[132]

To confirm conjugation between Gold Nano globe and oligonucleotide probes, infrared spectroscopy method used.[36]

Since the optical absorbance of gold nanoparticles when they are separated apart [shown in red], have been showed maximum light absorption at a wavelength of 520 nm and if the nanoparticles are accumulated [be seen in purple color].

**Optical absorption in the wavelength decreases:**

Therefore through using a spectrophotometer, the absorbance of gold nanoparticle alone, gold Nano probe after the conjugation and gold Nano probe with the PCR products were measured.

For this purpose, SPEKOL-2000-UV-1800 device that measure spectrum from 500 to 1100, were used.

**Results:**

For measuring small amounts of extracted DNA spectrophotometer was used and diluted of 1/25, 1/50 of extracted DNA was prepared and wavelength of A260 and A280 was read. The results of electrophoresis of DNA extracted from Bacillus cereus with Gram-positive extraction kit indicate healthy band with high purity the appropriate concentration of the extracted DNA was obtained and was used for PCR testing. Primers were designed for the specific nhC gene sequences of B. cereus.

Predicting the size of polymerase chain reaction product for these primers was 269 bp. And the used primers were linked to a specific part of the chromosome of the Bacillus cereus which was specific for this bacterium and consequently has the ability to identify Bacillus cereus.

Based on the obtained concentrations in the device, the PCR product concentration was calculated as follows:

\[
50 \times 0.66 + 25 \times 0.133 = 662.5/2 = 331.23 \mu g/ml
\]

The desired PCR product is for amplification of nhC gene is 269 bp that for the desired bacterium fragments at the exactly the size by gel electrophoresis was observed.

The molecular weight of the PCR product was calculated using the following formula:

\[
MW \text{ of SSDNA} = [An \times 313.2] + [Tn \times 304.2] + [Gn \times 329.2] + [Cn \times 289.2] + 79
\]

The nheC gene sequence was as follows and the number of its nucleotides are in the table below:

<table>
<thead>
<tr>
<th>5’tatggtatatcgctggtggtgtactttgtgtagcgttaataacatgccttgctggcggacc</th>
<th>3’</th>
</tr>
</thead>
<tbody>
<tr>
<td>4aacaaacatgactga</td>
<td>4aacgattgatgcagcaattacagcattgcaaaatatatcgaatca</td>
</tr>
<tr>
<td>atggtatatcaggggcaaatataata3’</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1-4:** obtained absorption and concentration of PCR product by spectrophotometer.

<table>
<thead>
<tr>
<th></th>
<th>PCR product with dilution of 1/50</th>
<th>PCR product with dilution of 1/25</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD at a wavelength of 260</td>
<td>0.180</td>
<td>0.560</td>
</tr>
<tr>
<td>OD at a wavelength of 280</td>
<td>0.099</td>
<td>0.198</td>
</tr>
<tr>
<td>Concentrations [micrograms per milliliter]</td>
<td>6/6</td>
<td>13/3</td>
</tr>
</tbody>
</table>
Table 2-4: the number of nucleotides in the PCR product.

<table>
<thead>
<tr>
<th></th>
<th>Number in the complementary strain</th>
<th>Number in the neC strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>40</td>
<td>61</td>
</tr>
<tr>
<td>C</td>
<td>61</td>
<td>40</td>
</tr>
<tr>
<td>T</td>
<td>100</td>
<td>68</td>
</tr>
<tr>
<td>A</td>
<td>68</td>
<td>100</td>
</tr>
</tbody>
</table>

The total molecular weight of the two strands is 66,339.6 gram per mol.

To perform the experiment, citrated gold nanoparticles were prepared the size of the nanoparticles was 10-15 nm and had the maximum absorption in wavelength of 520 nm and were seen red. To estimate the amount of conjugation the difference of purified oligonucleotides from column with oligonucleotide present in the supernatant derived from the washing Nano probe gold oligonucleotide was calculated. Concentrations of probes 1 and 2 are in order 5.31 and 7.28, and the concentration of the non-conjugated probes derived from soluble supernatant of Nano probes 1 and 2 was in order 7.13 and 5.11, which is equal to the difference between them and conjugation rate is calculated as follows:

Probe 1 = 31.5-13.7=17.8
Probe 2 = 28.7-11.5=17.2

Based on subtracting the above, the amount of conjugation for probe 1 and 2 in order was estimated 56/50% and 59/93%.

Nano-probe test was assessed using a spectrophotometer. Then, using FT-IR also evaluated and the last test was observed using an electron microscope TEM.

**Discussion and conclusions:**

Bacillus cereus conventional diagnostic methods include culture, immunological techniques and methods used in molecular microbiology. Culture-based methods are the common methods in this bacterium diagnosis that are followed by the isolation and biochemical identification and serological identification. [2, 37]

However, the detection of this pathogen in food through standard culture methods in the low contamination level [100> CFU/g], is difficult. [38]

Being time-consuming and laborious traditional method of cultivation is considered a serious problem in food industry applications. [1, 2, 3, 4]

Also, at least five days for the detection of Listeria species and about 10 days for the detection of Bacillus cereus through confirmatory test are required. However, the urgent measures about this contamination should be done this is especially true in the case of the short term foods storage such as meat and dairy products is important. [7]

On the other hand, May be due to the presence of damaged cells that can’t be cultured on selective medium, the presence of this bacteria seems to be ignored and in the presence of desired pathogen in food false-negative results were obtained. [5]

In addition to conventional culture-based methods, there are other methods to detect these pathogens, such as immunological assays such as ELISA and magnetic immunoassay [IMS] method. [5, 39]

These methods show better results than the conventional culture but to provide a sufficient number of bacterial cells for this test require an enrichment step which the stage is time consuming. [40]

In addition to the above methods, there are molecular detection methods such as PCR and Real-time PCR and RT-PCR. [41, 42]

In other words, most of the common diagnostic methods that some of them have been mentioned above, are relying on a primitive culture that it tends to slow down the process of identifying. [43]

In recent years, the use of nanoparticles, especially metal nanoparticles in biomedical research has expanded. Its advantage in the diagnosis and treatment is due to their unique properties, including small size, high surface area to volume ratio, high reactivity with living cells, stable at high temperatures and excessive transfer to cells. Typically, metal nanoparticles on study are including gold, silver, titanium oxide and iron. [44]

The present approach combines functionalization of gold nanoparticles with unique optical properties; it is done with Oligonucleotides with high diagnostic power and creates Composite materials which have a lot of application in medical diagnosis and are used in diagnostic tests.

Conjugation of biological molecules is done through nanoparticle that Biological recognition is based on nanotechnology. [45]

Colorimetric method based on Nano-probe has a sensitivity of 94/7% and while the colorimetric method based on non-functionalized gold nanoparticles has a sensitivity of 89/15% and this indicates that sensitivity of our study method is above the non-functionalized gold nanoparticle-based colorimetric method. [46, 47]

By optimizing this method it is possible A) create a new protocol for rapid detection of food contamination with Bacillus cereus B) increase the specificity and specificity of this method compared to other methods for detection of Bacillus cereus
In recent years, the use of metallic nanoparticles in biomedical research has expanded. [48]
One of the applications of nanotechnology is in the food pathogen detection which is a rapid and sensitive diagnostic method and to ensure food security is suitable. [49]
Using a colorimetric method, this is based on using a single-stranded probe and non-functionalized nanoparticles for the detection of DNA which is amplified through PCR. [50]
But there are newer methods that an oligonucleotide Nano probe with high diagnostic ability is used materials that are used in medical diagnosis and are used in diagnostic tests. [51]
Or the first time in 1991 Tsen and et ai used some special parts of Salmonella as oligonucleotide probes for the detection of Salmonella. [52]
In 1993 Hegner and et ai for the first time reported a method for binding DNA fragments using thiol on the surface of gold nanoparticles and the above combination in buffer solutions, took picture through atomic force microscopy [AFM] and demonstrated that the connections made between DNA molecules and Thiol gold nanoparticles are stable connections. [53]
Mirkin and et ai stated using a series of target oligonucleotide the oligonucleotide using two different nano probes and based on colorimetric were detectable.
Alghanian and et ai made a colorimetric sensor based on target DNA hybridization with its complementary oligonucleotide in which Gold nanoparticle surface using thioled oligonucleotide probes modified that the above probe, were hybridized with target DNA and leads to changing the suspension color. [55]
Mirkin and et ai first reported the creation of nano frame based on Gold nanoparticle that thioled Oligonucleotides were accumulated on the surface of gold nanoparticles. Taking advantage of the unique optical and electrical properties of the DNA conjugations gold nanoparticles, further research on the highly sensitive detection of DNA and proteins were conducted. [56]
Lee and et ai generated conjugates of DNA containing three thiol and gold nano particles with high stability and indicated that three thioled oligodeoxyribonucleotide can be used for stabling particles greater than 30 nm that has diagnostic use. [57]
Using unmodified gold nanoparticles colorimetric method for the detection of pathogens by Lee and Rodberg first was done in 2004 and today due to the no need for sophisticated and expensive equipment is a fast and easy way to detect pathogens. Huixiang and et ai Using electrostatic interactions with gold nanoparticles could recognize DNA sequences colorimetric method quickly and efficiently. [58]
Hioksang le and Lois Rodberg identified the DNA sequence based on Electrostatic interactions with gold nanoparticles. They found that single-stranded and double-stranded oligonucleotides have several trends to attracting gold nanoparticles in colloidal solution and gold nanoparticles in the presence of double-stranded DNA accumulates and change the color into purple. That was visible to the naked eye. This procedure does not require the covalent functionalization of gold nanoparticles, the probe and the target DNA. [46]
Calabretta and et ai created conjugations of gold-protein E.coli lac I Nanocrystalline with DNA sequence and concluded that through directing the conjugation use of alternative thiol in biomolecule area can be minimize the Inhibition of binding [59]
Hioksang le and et ai conducted experiment absorption difference between single-stranded and double-stranded DNA based on non-functionalized gold nanoparticles in a colloidal solution. In this experiment single-stranded DNA is absorbed by negatively charged gold Nano particles. While the double-stranded DNA is not able to connect which is due to differences in electrostatic properties And gold nanoparticles in the presence of double-stranded DNA accumulates and change the red color into purple. [60]
Machin[2009] investigated the Properties and stability of gold nanoparticles functionalized with thioled single-stranded DNA through using XPS and UV-Vis absorption method and concluded that Loading of single-stranded DNA on gold nanoparticles, is not significantly affected by the salt concentration. [61]
Conde and et ai used old nano-probes for measuring specific RNA to detect cancer cells and indicated that due to the cheap and easy method, it could be a good alternative to many common procedures in the diagnosis of cancer. [62]
Veigas and et ai used Gold nano-probes to detect specific SNP within the RNA polymerase beta subunit [locus rpoB] Mycobacterium tuberculosis, which is responsible for 95% of the bacteria resistant to rifampicin. [63]
Hosub lee and et ai could recognize the mutation in the growth factor receptor with colorimetric method using gold nanoparticle accumulation. [46]
Fan X., Xiaolei and et ai could detect DNA small molecules, proteins and ions through using non-functionalized gold nanoparticles and conjugated polyelectrolyte with colorimetric method. [64]
Gold nanoparticle-based colorimetric assay for the rapid detection of Salmonella species in food using single-stranded DNA probes and non-functionalized by gold nanoparticles, was done by Dinesh Parsad and et ai. In detection of PCR amplify DNA through colorimetric method; the sensitivity and specificity are in order 89/15% and 99/4%.
Colorimetric method is 10 times more sensitive than diagnosis based gel with the same concentration of DNA used for the analysis. [50]

Yanli zu and et al diagnosis Lysine through accumulation of gold nanoparticles by the colorimetric method. They found that simple and reliable and sensitive Colorimetric method for the detection of lysine is in acidic PH = 3. And change the color of gold nanoparticles after adding lysine for 30 min at room temperature can be seen with the naked eye. [65]

Gill and et al have done the Diagnostic Nano method for colorimetric detection of Mycobacterium Tuberculosis sr RNA 16. In this experiment RNA-DNA hybridization through Colorimetric method with gold nanoparticles accumulation can be detected in which mycobacterium is diagnosed in less than an hour. The result of clinical samples showed 94/7% of the sensitivity, this method is a rapid method for the detection of Mycobacterium in clinical samples. [43]

We can conclude from the investigation that since nheC gene is a chromosomal gene, so in this study for the first time through colorimetric method based on designed gold oligonucleotide Nano probe which were the complementary to the inner part of the product nheC gene amplification. Used as a fast method with high sensitivity and specificity for the detection of Bacillus cereus.

**REFERENCES**


[10] Heikl lou, Z., Biological production of gold nanoparticles by the fungus isolated from Ahar Copper mine & Andilian gold mine.


Effects of shape and charge of colloidal gold nanoparticles in colorimetric determination of DNA:


