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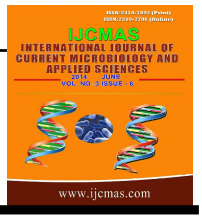

Original Research Article

Detection of *E.coli O157:H7* in feed samples using gold nanoparticles sensor

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Keywords

Gold Nanoparticles, Sensor, *E.coli O157:H*, Feed & Detection

A B S T R A C T

Food-borne illness is resulting from the consumption of contaminated food by bacteria or other microbes. Animal by-products, such as feed supplements, may also transmit pathogens as *Escherichia coli O157:H7* to feed animals, also can cause serious food poisoning in humans.Traditional detection method of bacteria takes 2-5 days for a final characterization, it remains evident of time-consuming. Rapid, selective and sensitive detection technologies for pathogenic bacteria are critical in clinical diagnosis, disease control, feed and food safety. Nanotechnology, as a new enabling technology, has the potential to revolution in agriculture, food and feed systems. Gold nanoparticles (AuNPs) used as a biosensor for it is ability of combination with a variety of techniques. In present study, detection of *E.coli O157:H7* as sensitive colorimetric method was developed using conjugated gold nanoparticles anti-*E.coli O157:H7* sensor. The key point of gold nanoparticle-based visual detection assay is to control dispersion and aggregation of colloidal nanoparticles by targets of interest *E.coli O157:H7*. The existence of the target molecules can be translated into optical signals and monitored by the naked eyeresulting in a dramatic color change from red to blue.Therefore, this developed method is a novel, on-site and takes few minutes from bacterium binding to detection and analysis and could be applied for wide range of practical applications.

Introduction

Food safety continued to draw the attention of consumers, food manufacturers, and producers. Food-borne illness is resulting from the consumption of contaminated food. Food infection refers to the presence of bacteria or other microbes which infected the body after consumption.

Food toxication refers to the ingestion of toxins contained within the food, including bacterially produced exotoxins, which can happen even when the microbe that produced the toxin is no longer present or able to cause infection. Bacteria are a common cause of food-borne illness.

Infectious disease, commonly caused by bacterial pathogens, is now the world's leading cause of premature death and third overall cause behind cardiovascular disease (Basu*et al.*, 2004).

E.coli is the head of the large bacterial family, *Enterobacteriaceae*, the enteric bacteria, which are facultatively anaerobic Gram-negative rod-shaped bacterium that live in the intestinal tracts of animals in health and disease. Commonly found in the lower intestine of warm-blooded organisms (endotherms). *E. coli* are not always confined to the intestine, and their ability to survive for brief periods outside the body makes them an ideal indicator organism to test environmental samples for fecal contamination. The bacteria can also be grown easily and its genetics are comparatively simple and easily manipulated or duplicated through a process of metagenics, making it one of the best-studied prokaryotic model organisms. Food poisoning caused by *E. coli* is usually caused by eating unwashed vegetables or undercooked meat. *E.coli* O157:H7 is emerging as the most aggressive one among hundreds of strains the bacteria, because of its capability to produce a toxin causing hemolytic uremic syndrome (HUS), kidney failure if not detected quickly, diarrhea and death in the elderly that is unpleasant in healthy adults and is often lethal to children in the developing world (Todar, 2008). So, *E.coli* O157:H7 has been chosen for performance of this study.

Rapid, selective and sensitive detection technologies for pathogenic bacteria are critical in clinical diagnosis, disease control, environmental monitoring and food safety. Conventional identification methods take 2–3 days for results, and up to 7–10 days for confirmation. It evident that steps are the consuming time of waiting for cultures to grow which detracts the analysis process efficiency, while fairly accurate (Tu*et al.*, 2009). Recently, many attempts have been made to improve the sensitivity of detecting pathogenic microorganisms, without the need for target amplification and enrichment. Nanotechnology one of this attempts which defined as the manipulation of matter with at least one dimension sized from 1 to 100 nanometers, one nanometer is a billionth of a meter. Application of nanotechnology to detect pathogens from such complex systems presents numerous challenges. Nanotechnology, as a new enabling technology, has the potential to revolution in agriculture and food systems, new tools for molecular and cellular biology and a new material for pathogen detection (Bhunia, 2008).

Antibody-antigen binding consider the basic idea of Immunosensors detection that have been used successfully for the of microorganisms detection (Ricci *et al.*, 2007). Biosensor-based technologies have been increasingly used in the development of methods to sensitively detect food-borne pathogens. Biosensors are devices that detect biological or chemical recognition complexes in the form of antigen–antibody, enzyme–substrate, or receptor–ligand, placed in proximity to a transducer that generates a signal. The application of biosensors for pathogen detection from food samples are challenging because of the complex nature of food matrices.Optical biosensors appear to be the most widely used sensors for food-borne pathogens because of their sensitivity, available instrumentation, and relative ease of data interpretation (Anderson *et al.*, 2005). Perez (2013) has developed magnetic nanosensors for the rapid detection of microbiological agents

in complex media. Specifically, he has reported the sensitive detection of bacteria (*Mycobacterium avium* spp. *paratuberculosis* - MAP) in milk and blood within 30 minutes, using dextran-coated iron oxide nanoparticles conjugated with antibodies that recognize surface proteins found on the bacteria.

The use of AuNPs in biosensors is a relatively new area of research. Nevertheless, literature already shows numerous examples of incorporating NPs into biodevices. So far, there are nanobiosensors for the specific detection of biologically relevant molecules and sometimes for the detection of infectious agents. Also, AuNPs have attracted significant interest as a novel platform for various applications such as nanobiotechnology and biomedicine because of convenient surface bio-conjugation with molecular probes and remarkable plasmon-resonant optical properties.

AuNPs exhibit remarkable optical properties and could prove useful in sensitive biosensing applications (Biradar*et al.*, 2012). AuNPs have good biological compatibility, conducting capability, and high surface-to-volume ratio. AuNPs have been employed in several techniques, as DNA sensing, enzyme biosensing, and electrochemical sensing. Antibodies can be immobilized onto AuNPs, therefore AuNPs have been used to improve the sensitivity of bacteria detection (Guoand Wang, 2007).

The aim of the present study is using bioconjugated antiserum gold nanoparticles (AuNPs) in developed a bioassay for accurate detection of *E.coli O157:H7* in feed sample within few minutes without any feed sample enrichment.

Materials and Methods

Thepresent study concerned with accomplishment of traditional micro-biological testes and AuNPs biosensor detection of *Escherichia coli 0157:H7* in yellow corn sample, after inoculation 4 types of microorganism (*Escherichia coli 0157:H7, Bacillus cereus, Staphyllococcusaureus*and *Salmonella typhimurium)* in this sample to prove that the AuNPs biosensor is characteristic for the detection of *Escherichia coli 0157:H7*withoutallotherbacteria.

Microorganisms were obtained from culture collection of Cairo MIRCEN, Agricultural Studies and Consultation Center, Faculty of Agricultural, Ain Shams University. All strains were morphologically and physiologically checked, subculture monthly, maintained on nutrients agar slant and kept in refrigerator at 4ºC. Tetrachloroauric acid (III), sodium citrate tribasic dehydrate , polyethylene glycol, *E.coli O157:H7* antiserum (SA Scientific – USA),phosphate buffer saline, potassium chloride, sodium chloride, sodium phosphate, potassium phosphate were used in this study. All chemicals were commercially purchased from Sigma-Aldrich and were of analytical grade.

Sample preparation

Yellow corn sample was obtained from Regional Center for Food and Feed (RCFF), Agricultural Research Center, Ministry of Agriculture, Egypt, placed on autoclave at 121ºC for 15 minutes 1.2 atmospheric pressure to get rid of any kinds of bacteria under the present study. One hundred gram of sample divided into equal 4 parts (25 g/part), every part was inoculated with each of the tested microorganism previous sub-cultured on

nutrient agar slants and incubated at 37 ºC for 24 hours. Afterword heavy inoculum from the subculture was diluted in brain heart infusion broth medium to obtain culture density enough for inoculating of the yellow corn sample in density of about 10^6 cells/gm. Counts of each microorganism were traced and recorded. Prepare series dilution (10^1-10^6) from all each previous microorganism.

Synthesis of colloidal gold nanoparticles

Monodispersed 40 nm colloidal gold nanoparticles generated by the conventional reduction of tetra-chloroauric acid with tri-sodium citrate according to method described by Zhao (Zhao *et al*., 2010). The size of the colloidal particles (40 nm) depends on the rate of nucleation and crystal growth by controlling the reduction of the tetra-chloroauric acid. In brief; 50 ml of 0.01% (W/V) aqueous solution of gold (III) chloride trihydrate was heated to boiling with stirring, rapidly add 300 μL of 1% trisodium citrate solution, keep solutions boiling for about 15 min till the color change to bright red and let it cool at room temperature.

Characterization of gold nanoparticles instruments

UV-Vis spectrophotometer, (Cary 5000, Varian, UK) monitoring AuNPs from 400 to 800 nm with a path-length of 10 mm at 12 nm/s scanning speed and 1 nm bandwidth (Todebush*et al.*, 2002).Transmission electron microscopy (TEM), Philips CM200- Netherland voltage of 120 KV. Samples were prepared by placing a droplet of the colloidal solution onto a carbon-coated copper grid and were allowed to dry for 45 minutes.Zeta Potentials(Malvern, UK) with rang (-200:200mV), used for AuNPs charge measurement (Ijeh, 2011).

Conjugation of gold nanoparticles and *E.Coli O157:H7* antibodies

The conjugation between AuNPs and antibody prepared by adsorption of antibodies on AuNPs surface which is pH and antibodies concentration dependent (Horisberger, 1990).Thus, the optimal conditions of binding have to be determined for each antibody to be tagged. Inadequate conditions will lead to flocculation and precipitation of AuNPs. Conjugation of *E.coliO157:H7*antibodies withgold nanoparticles was done as described by Huang (2006).

Detection of *E.coli O157:H7* using conjugated AuNPs sensor

1- Control sample, shacking of 0.5 ml saline-peptone solution buffer with 2ml conjugated AuNPs anti-*E.Coli O157:H7*.
2- 0.5 ml from each series dilutions of*E.coli O157:H7* (10^{-1} - 10^{-6}) added to 2ml conjugated AuNPsanti-*E.Coli O157:H7* with shacking.
3- Repeating above steps with other series dilutions of*Salmonellatyphimurim*, *Staphyllococcusaureus* and *Bacillus cereus.*
4- Samples were monitored using UV visible spectroscopy.

Conventional Detection of *E.coli 0157:H7*

Culture media for detection (Iso, 2001) was sorbitol Macconkey agar (Difco, 1989). The enrichment media was modified tryptone soya broth (MTSB+N) (MTSB) (Bolton *et al*., 1995) and the subculture on medium containing cefixime. Biochemical confirmatory testes of *E.coli 0157:H7*. Representative colonies on a suitable plate were transferred to nutrient agar slants and the

cultures were examined through the following tests: gram staining, catalase test, sugar fermentation test, lactose test, indole test, nitrate test and serological test for *Escherichia coli 0157:H7*.

Results and Discussion

Characterization of gold nanoparticles

UV-Vis spectrophotometer

Gold nanoparticales have optical properties characterized by their Plasmon absorbance band in the visible region of the electromagnetic wave particles have peaks at 530 nm on size 40 nm (Figure, 1). The obtained result was in accordance with previously published by Huang (2006) with absorption peak at 530 nm with Gaussian distribution indicating formation of spherical AuNPs with no aggregation of size that indicated uniformity and excellent dispersion of colloidal gold nanoparticles (Perezjuste, 2005).

Transmission electron microscopy (TEM)

The high resolusion-TEM (HR-TEM) image of AuNPs was synthised by citrate method whith monodisperse spherical shape with average size 40 nm (Figure, 2). AuNPs were clearly evident and that particles appeared spherical and uniform in size demonstrating homogeneity and monodispersity

Zeta Potentials

Zeta potential measurement of the prepared colloidal AuNPs (40 nm) by citrate methods was -32 mV (Figure, 3)which confirmed the negative charges formation on the surface of AuNPs.Negative charge result from citrate which plays the role of both a reducing and a stabilizing agent that repulsion between AuNPs that forestall the aggregation of AuNPs (Todebush *et al*., 2002).If the particles have low zeta potential (less than -25 values) then there was no hindrance technique for particles flocculating.

Antibody gold nanoparticles conjugation

HR-TEM image observed that the antibody- AuNPs conjugation were still monodisperse and average size of the particles was 40 nm, which was propitious for strong signal generation in immuochromatography test strip. AuNPs is clearly evident that particles are spherical and uniform in size demonstrating homogeneity and monodispersity which confirm no aggregations between AuNPs (Figure, 4).

The conjugation technique of AuNPs-antibodies was formed through electrostatic interactions of antibodies with charged particles surfaces. A great deal of information is known about the nature of bonding between gold nanoparticles and antibodies. It was suggested that electrostatic interaction between antibodies and AuNPs depends on three separate types of interactions but dependent phenomena may take place(Figure, 5): (a) The hydrophobic attraction between hydrophobic parts of the antibody and the gold surface, (b) The ionic attraction between the negatively charged surface of the gold particles and the positively charged antibody due to present of amino acids and the N-terminal, and (c) The dative binding between the gold conducting electrons and free sulfhydryl groups of the antibody (Ljungblad, 2009andIjeh, 2011).

Detection of *E.coli O157:H7* in tested yellow corn sample inoculated with *E.coli O157:H7* using AuNPs sensor

After adding different series dilution (10^1 - 10^6) of *E.coli O157:H7* to conjugated AuNPs-anti-*E.coli O157:H7*. There was a change in solutions color from red to blue within minutes to all different series dilution except the color of control solution (Free bacteria) did not changed. This blue color formation has been seen clearly by naked eye (Figure, 6). By measuring these series dilutions and control using spectrophotometer, it was found that the final sheet spectra showed that the wave length of control peak was 530±5 nm, while the others series dilutions peaks were 645±5 nm (Figure, 7). When nanoparticles aggregated, the wavelengths of light absorbed changed from red to blue from 600 to 700 nm. It leads to the formation of clusters of particles, or even a monolayer of particles on a surface (Todebush*et al*., 2002).

From previous results, it can be concluded that the blue color output in testing dilution samples due to conjugate between *E.coli O157:H7* and specific site of its antibodies. Moreover, this correlation (*E.coli O157:H7*-antibody link) occurrence of aggregation to gold nanoparticles.This color change by aggregation (Haichao *et al*,. 2012).

Aggregation of gold nanoparticles

Nanoparticles have a high surface area to volume ratio; the Plasmon frequency is exquisitely sensitive to the dielectric (refractive index) nature of its interface with the local medium. Any change to the surroundings of nanoparticles (surface modification, aggregation, medium refractive index, etc.) leads to colorimetric changes of the dispersions Figure (8). Sticking particles together changes their color in a similar way to the change of color associated with changing size or shape of the particles (Murphy*et al*., 2008).

Detection *E.coli O157:H7* of in tested Yellow corn sample inoculated with *Salmonella typhimurium, Staphylicoccus aureus* and *Bacillus subtitles* using AuNPs sensor

Detection of different types of bacteria (*Salmonella typhimurium*, *Staphylicoccus aureus* and *Bacillus subtitles*).By adding previous series dilutions (10^1 - 10^6) to AuNPs-anti-*E.Coli O157:H7*. The result observed that no change color in all series dilutions compared to the control sample which still red. These coloring outputs have been seen clearly by naked eye for *Salmonella typhimurium* (Figure, 9), *Staphylicoccus aureus* (Figure, 11), *Bacillus subtitles* (Figure, 13).

By measuring using spectrophotometer for previous different series dilution to all types of bacteria, the obtain spectra show that the wavelength of beaks were 530±5 nm for each control and series dilution.These peaks results confirmed that no correlation between either *Salmonella typhimurium* (Figure, 10), *Staphylicoccus aureus*(Figure, 12)or *Bacillus subtitles* (Figure, 14) with specific site of antibodies on AuNPs-anti-*E.Coli O157:H7*.

Convention detection of *E.coli O157:H7* in tested yellow corn sample

Detection of *E.coli O157:H7* using conventional methods (biochemical, physiological and serological tests) proved the presence of *E.coli O157:H7* in a yellow corn sample that have been

Figure.1 UV-Vis spectrum of AuNPs with absorption peak at 530 nm

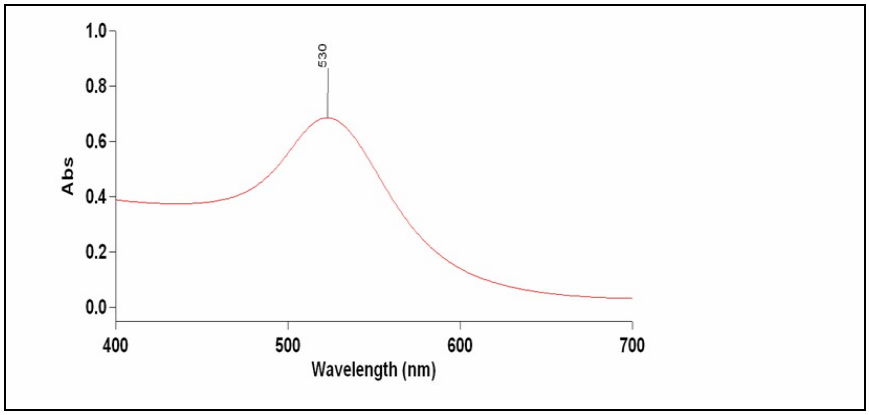


Figure.2 HR-TEM image of AuNPs with average size 40 nm.

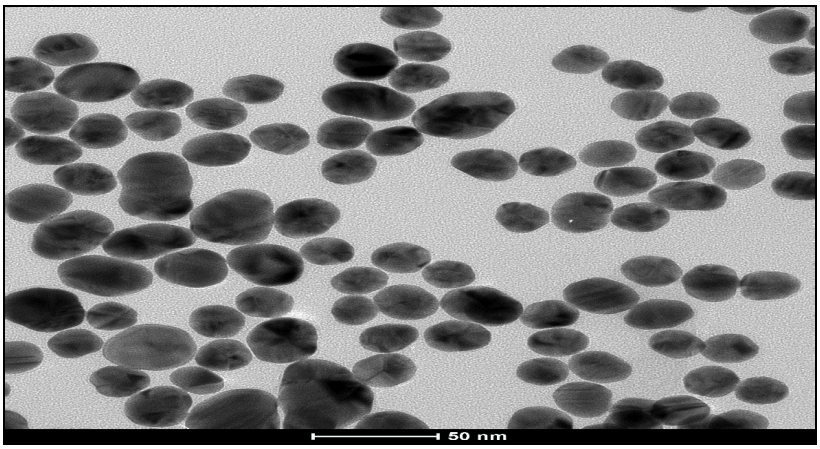


Figure.3 Zeta Potential of the gold nanoparticles has -32 mv

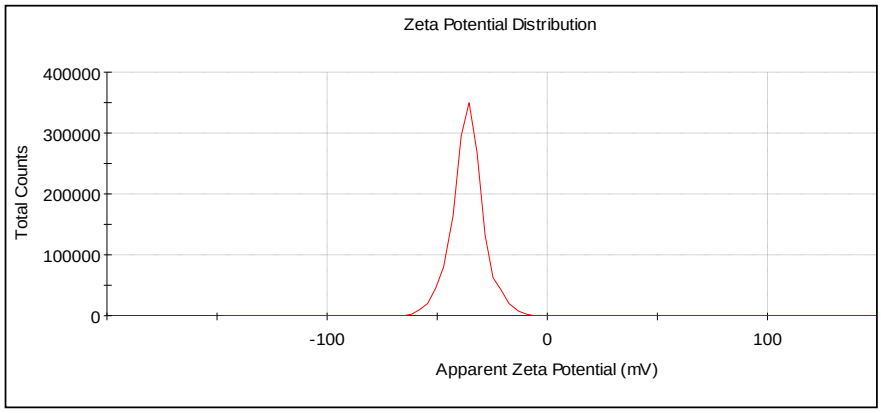


Figure.4 HR-TEM image AuNPs-antibodies conjugation

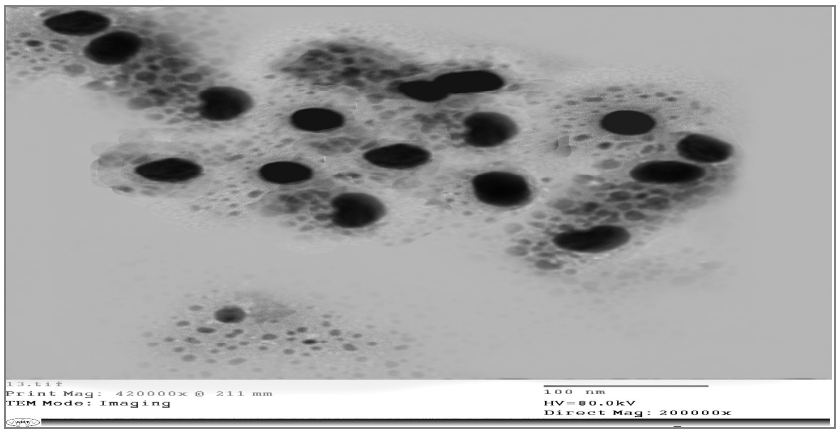


Figure.5 A schematic representation of conjugation process

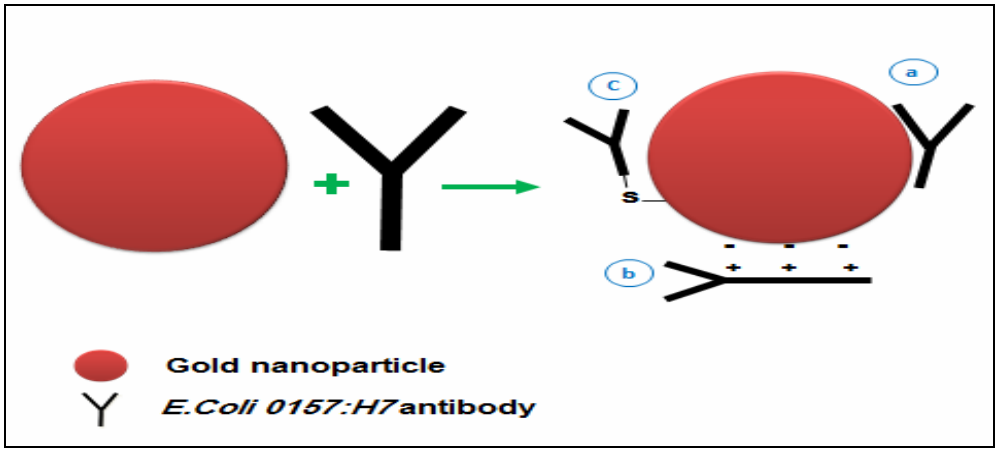


Figure.6 AuNPs-anti *E.coli O157:H7* after adding serial dilutions of *E.coli O157:H7*

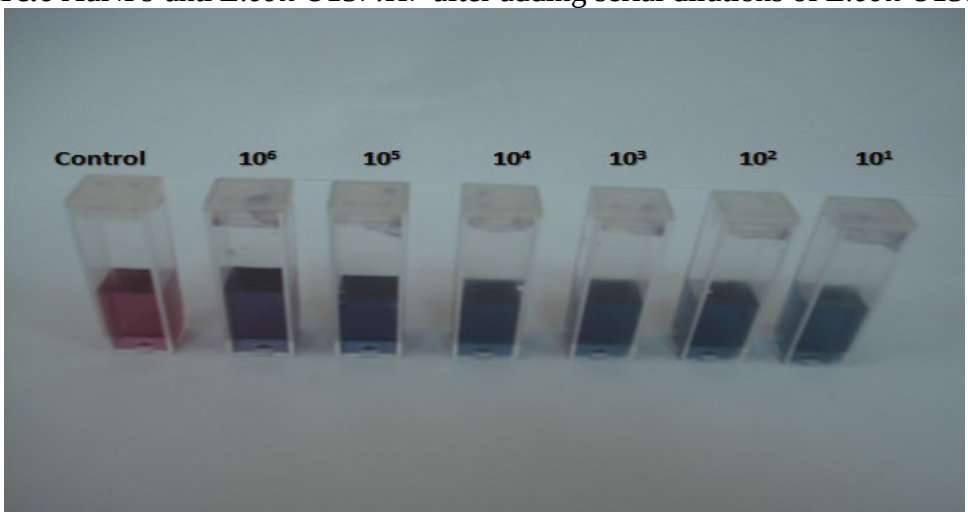


Figure.7 Spectra of AuNPs-anti *E.coli O157:H7* after adding serial dilutions of *E.coli O157:H7*

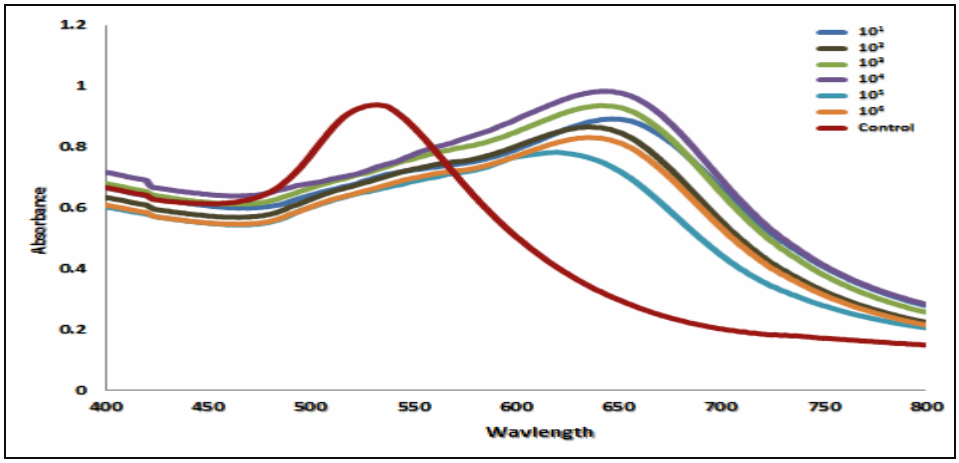
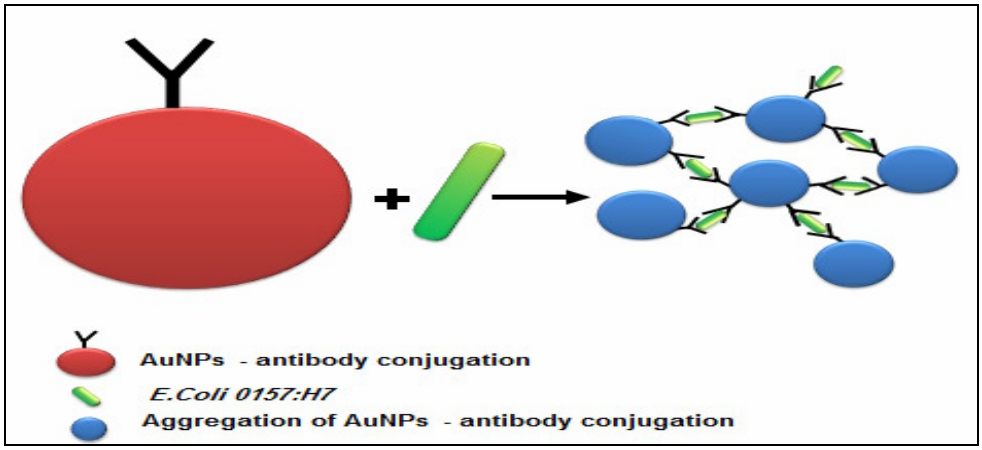


Figure.8 Aggregation of AuNPs-antibody conjugation



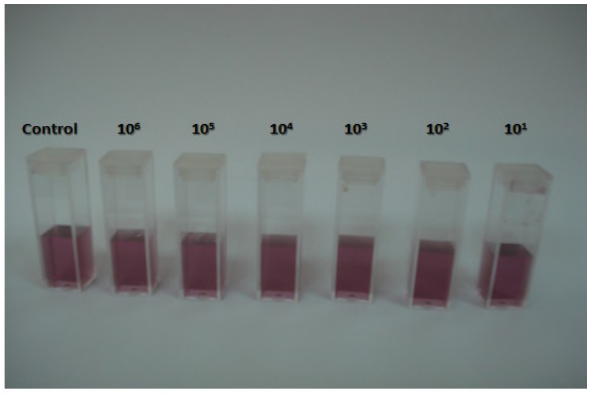


Figure.9 AuNPs-anti *E.coli O157:H7* after adding serial dilutions of ***Salmonella typhimurium***

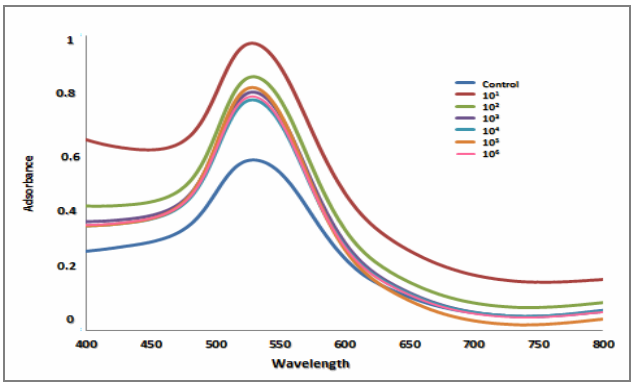


Figure.10 Spectra of AuNPs-anti *E.coli O157:H7* after adding serial dilutions of ***Salmonella typhimurium***

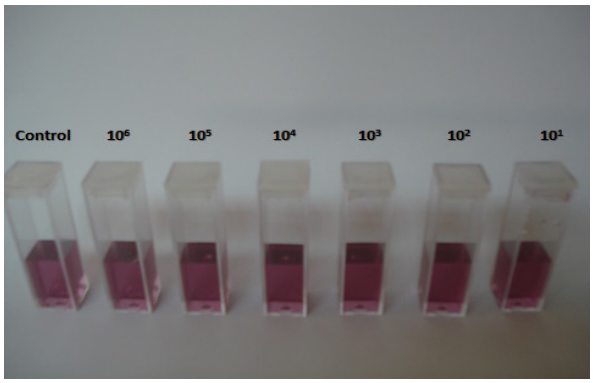


Figure.11 AuNPs-anti *E.coli O157:H7* after adding serial dilutions of***Staphylicoccusaureus***

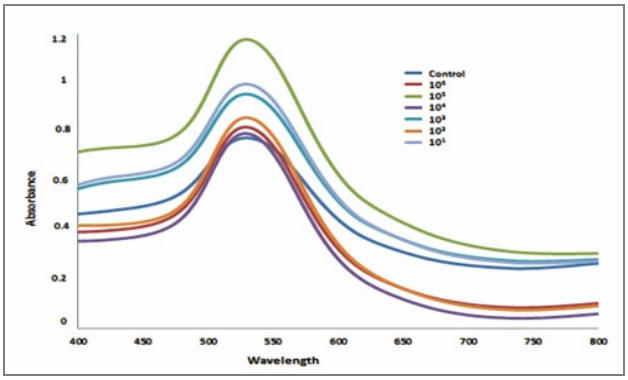


Figure.12 Spectra of AuNPs-anti *E.coli O157:H7* after adding serial dilutions of***Staphylicoccusaureus***

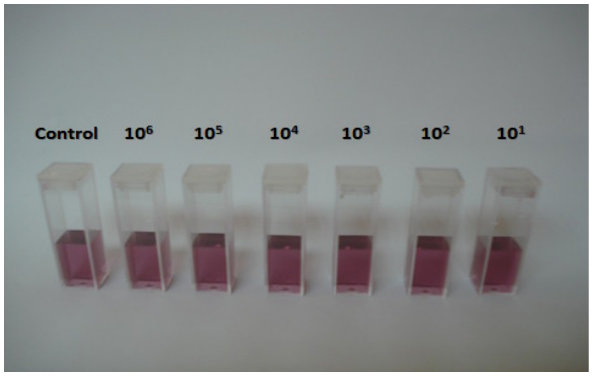


Figure.13 AuNPs-anti *E.coli O157:H7* after adding serial dilutions of ***Bacillus subtitles***

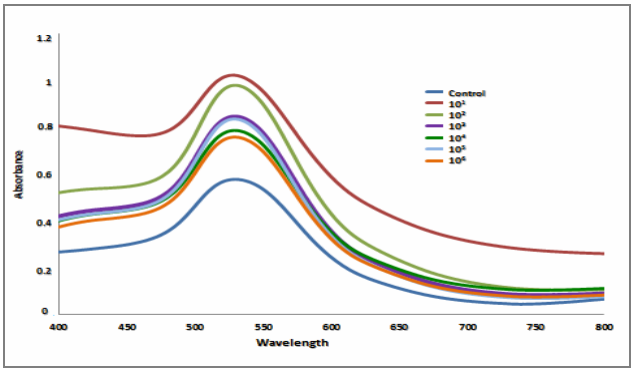


Figure.14 Spectra of AuNPs-anti *E.coli O157:H7* after adding serial dilutions of ***Bacillus subtilus***

inoculated with this bacterium which typical colonies are transparent and almost color less are with pale yellowish-brown appearance, beside to confirmation tests also test is carried out by examining suspected colonies culture on (CT-SMAC) modified sorbitol maconkey agar. Test for slid agglutination. Test by using specific sera (Difco 229701) (Forbes, *et al*., 2007).), except another parts of the corn sample that inoculated with (*Salmonella typhimurium*, *Staphylicoccus aureus* and *Bacillus subtitles*).

From previous results could be concluded that the detection of *E.coli O157:H7* by traditional methods confirmed the detection results of the AuNPs sensor, as a colormetric method in detection of *E.coli O157:H7* in yellow corn sample.

Instead of traditional detection method which takes few days for enrichment and detection**,** it could utilize of AuNPs in colorimetric detection inherits the simplicity of conventional AuNP-based sensing strategies because it can be read out by the naked eye, does not require the assistance of complicated instruments and it take few minutes compeared to traditional methods**.** The change of color from red to blue output in testing dilution samples due to binding between *E.coli O157:H7* and specific site of its antibodies

which conjugate with AuNPs. Moreover, this correlation (*E.coli O157:H7*-antibody link) occurrence of aggregation to gold nanoparticles**.** These advantages make it useful for rapid, sensitive, convenient detection and most likely possess great prospects in the future.

References

Anderson, G.P. and Taitt, C.A.R. (2005): Biosensor-based detetction of foodborne pathogens. ‘‘Foodborne Pathogens: Microbiology and Molecular Biology’’Caister Academic Press, Norfolk. P. 33–50.

Forbes, A.B., Sahm, F.D., Weissfeld, S.A. and Trevino, A.E. (2007): Bailey and Scott□s diagonstic microbiology. 12th international edition, MOSBY elsevier publisher, PP.1056.

Basu, M., Seggerson S., Henshaw J., Jiang J., Rocio, A.C., Clare L., Boyle P., Miller A., Pugia M. and Basu S. (2004): Nano-biosensor development for bacterial detection during human kidney infection: Use of glycoconjugate-specific antibody-bound gold nano wire arrays (GNWA). Glycoconjugate Journal. 21 (8-9):487-496.

Bhunia, A.K. (2008): Biosensors and bio-based methods for the separation and detection of foodborne pathogens. Advances in food and nutrition research, 54:1-44.

Biradar D., Lingappa K. and Dayanand A. (2012): Antibacterial activity of nanogoldparticles synthesized by *Bacillus Sps*. Journal of Ecobiotechnology, 4(1): 43-45.

Bolton, F.J.,Croziev, L. and Wiliamson, J.K. (1995): Optimisation of methods for isolation of *Escherichia coli O157:H7* from beef burgers. PHLS Microbilogy Digest.,12: 67-750.

Guo, S., and Wang,E. (2007): Synthesis and electrochemical applications of gold nanoparticles. Analytica Chimica Acta.598:181-192.

Haichao, S., Qiang, M., Kun, S., Tao, L., Huanshun, Y., Shiyun, A. (2012): Gold nanoparticles as colorimetric sensor: A case study on *E.coli O157:H7* as a model for Gram-negative bacteria. Sensors and Actuators B: Chemical.161: 298– 303.

Horisberger, M. (1990): Quantitative aspects of labelling colloidal gold with proteins in immunocytochemistry. In Trends in Colloid and Interface Science *IV SE - 32*; Zulauf, M.; Lindner, P.; Terech, P., Eds.; Steinkopff. 81: 156–160.

Huang, X. (2006): Gold nanoparticles used in cancer cell diagnostics, Selective photothermal therapy and catalysis of NADH oxidation reaction. Ph. D. thesis. Georgia Institute of Technology. P. 143.

Ijeh, M. (2011): Covalent gold nanoparticle antibody conjugates for sensitivity improvement in LFIA. Ph. D. thesis. Informatics and natural sciences. Faculty of Hamburg University. P. 147.

Iso (2001): Microbiology of food and animal feeding stuffs preparation of test samples, initial suspension and decimal dilutions for microbiological examination.

Ljungblad, J.(2009): Antibody-conjugated gold nanoparticles integrated in a fluorescence based biochip. Ph.D. thesis. Department of physics, Chemistry and Biology, Linköpings University, Sweden. P. 53.

Murphy C.J., Gole A.M., Stone J.W., Sisco P.N., AlkilanyA.M , Goldsmith E.C., and Baxter S.C. (2008):Gold nanoparticles in biology: Beyond toxicity to cellular imaging. Acc.

Chem. Res., ASAP. DOI: 10.1021/ar800035u.

Perez, J.M. (2013): Pathogen and Toxin Detection. Nano Science Technology Center. jmperez@ucf.edu

Perezjuste, J. (2005). Gold nanorods: Synthesis, characterization and applications. Coordination Chemistry Reviews, 249 (17-18):1870–1901.

Ricci, F., G. Volpe, L. Micheli, and G. Palleschi. (2007): A review on novel developments and applications of immunosensors in food analysis. AnalyticaChimicaActa.605:111-129.

Todar K. (2008): Pathogenic *E. coli*. Online Textbook of Bacteriology. Ph.D. thesis. Department of Bacteriology. University of Wisconsin. www.textbookofbacteriology.net

Todebush, M. P., Camden J., Roden J. and Schatz G.C. (2002): Theoretical Analysis of GoldNanoparticles. Department of Chemistry. Northwestern University.

Tu, S., S. Reed, A. Gehring, Y.He, and G. Paoli. (2009): Capture of *Escherichia coli* O157:H7 using immuno magneticbeads of different size and antibody conjugating chemistry. Sensors.9:717-730.

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