Mini Abstract

Bovine mastitis a major disease affecting dairy cattle worldwide, results from the inflammation of the mammary gland. In this study we target *Staphylococcus aureus* because it is one of the most significant contagious pathogens. There are many procedures for diagnosis of *S. aureus* which require expert technicians and require complicated procedures. The colorimetric detection of nucleic acid using gold nanoparticles (GNP) is widely used due to the extraordinary importance of nucleotide sequences in biological analyzes as well as the characteristics and advantages of using GNP in detection, such as simplicity, easy preparation, low cost and time saving.

Taking the advantage of colorimetric detection, our technique was plan to detect *S. aureus* genomic DNA using GNP conjugated probe. The *nuc* gene specific probe was designed because the nucleotide sequence of *nuc* gene poses apparently unique sequences of the *S. aureus*. Thiol group is most widely used as interaction to achieve the stable conjugation between DNA oligonucleotides and GNP. So, 5' thiol modified probe was used for conjugation with GNP. GNPs synthesized by citrate reduction of gold chloride are stable in solution and show λ_{max} at 515.2 nm for GNP. Upon conjugation with probe λ_{max} shifted from 515.2 nm to 521 nm indicating successful conjugation of probes to GNP. Colour change from red to purple was observed by decrease in pH upon addition of 130 mM HCl to conjugated GNP. While below 130 mM HCl concentration no colour change was observed. Initially, the GNP oligo probe assay was optimized for visual detection using 22 base complementary DNA sequences. It was found that optimum temperature for hybridization is 60°C for GNP probe and the target DNA. Colour change was observed from red to purple when hybridised GNP oligo with complementary DNA due to aggregation of GNP. The sensitivity of this assay was from sample containing 3.49 µg/ml to 0.35 ng/ml of PCR amplicon of *nuc* gene and from 12.59 µg/ml to 1.26 ng/ml of genomic DNA. In order to determine the specificity, The GNP oligo probe assay was applied to the genomic DNA isolated from *S. aureus*, *Escherichia coli*, *Aeromonas hydrophila* and *Clostridium perfringens* and observed that the solution containing *S. aureus*, genomic DNA remain red, whereas the solution containing DNA of other bacteria turned purple after addition of HCl. Thus, this assay can differentiate *S. aureus* and non *S. aureus* genomic DNA by naked eye without using any sophisticated instrument. This method is rapid and convenient, and can be accomplished within 30-40 min and a significant colour change that is easily recognized by the naked eye. This method is suitable for detection of detection of *S. aureus* genomic DNA from clinical sample.

DEPARTMENT OF VETERINARY BIOCHEMISTRY
BIHAR VETERINARY COLLEGE, PATNA - 14
(Bihar Animal Sciences University, Patna, Bihar)

CERTIFICATE - I

This is to certify that the thesis entitled **" Evaluation of gold nano particle conjugated *nuc* gene oligonucleotide probe for rapid detection of *Staphylococcus. Aureus* "** Submitted in partial fulfillment of the requirement for the award of the degree of **Master of Veterinary Science** in the discipline of **Veterinary Biochemistry** of the faculty of Post-Graduate Studies, Bihar Animal Sciences University, Patna, Bihar is a bonafide research work carried out by **Dr. Ayush Ranjan, registration No.: VM0020/2019-20** under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigation have been fully acknowledge

Place: _____

Date: _____

(Ajeet Kumar)
Major Advisor

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CERTIFICATE – II

This is to certify that the thesis entitled **"Evaluation of gold nano particle conjugated *nuc* gene oligonucleotide probe for rapid detection of *Staphylococcus. Aureus*"** submitted by **Dr. Ayush Ranjan,** Registration No**.: VM0020/2019-20,** to the Bihar Animal Sciences University, Patna in partial fulfillment of the requirements for the degree of **Master of Veterinary Science** in the discipline of Veterinary **Biochemistry has** been approval by Student's Advisory Committee after an oral examination of the student in collaboration with an External Examiner

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1. Introduction

India is the largest producer of milk in the world and it accounts for 22% of the world milk production (Faye and Konuspayeva, 2020). Animal health is the mainstay of the speedily growing livestock and dairy industry. Dairy farming forms the second largest economic activity in India and over 90 per cent of milch animals are reared in unorganized sector by the small farmers and mastitis is an overwhelming disease upsetting their livelihood. (Sharma *et al*., 2004).

Mastitis is the most significant disease in dairy herds. Subclinical mastitis (SCM) in cows is more prevalent in India than clinical mastitis. Average loss of milk yield due to subclinical mastitis is up to 17.5% (Shaikh *et al*., 2019). Mastitiscauses perpetual economic loss to the farmer because of reduction in milk production and treatment costs (Sharma *et al*., 2007). Mastitis is a multietiologic multifaceted disease, in which inflammation of parenchyma cells of udder take placeand it is reflected by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissues. The mastistis causing bacterial species are *Escherichia Coli, Staphylococcus aureus, Streptococcus agalactiae, and Corynebacteriumbovis*etc

S. aureus causes one of the most common forms of chronic mastitis. The infection is spread at milking time when *S. aureus* contaminated milk from an infected gland comes in contact with an uninfected gland, and the bacteria enter the teat canal. The conventional identification of pathogen involves isolation and biochemical characterization, which is timeconsuming and laborious (Elsheshtawy*et al,* 2019). Nucleic acid-based methods such Ribotyping, Amplified Fragment Length Polymorphism, Randomly Amplified Polymorphic DNA, Polymerase chain reaction (PCR), Real Time PCR, Ligase chain reaction (LCR) are also used for detection of pathogen (Koskinen *et al*, 2010). Other sensitive methods like Liquid chromatography-mass spectrometry (LCMS), immuno-PCR, and Chemiluminescence method were developed. These methods in most cases requires highly specialized personnel, dedicated equipment and are time consuming (Martins *et al.*, 2019). There is need to develop rapid approaches, capable of providing on-site information concerning the infection.

Optical biosensors for rapid detection of pathogens are steadily gaining popularity due to its simplicity and sensitivity. Nanomaterials have exclusive biological and physiological properties that can be of great utility in creating new gratitude and transduction methods for

chemical and biological sensors as well as refining the ratio by diminishment of the sensor elements (Quintela*et al.,* 2019). Gold nanoparticles (GNP) possess distinct physical and chemical properties that make them excellent scaffolds for the fabrication of novel chemical and biological sensors. Surface plasmon resonance (SPR) provide unique optical properties to GNP (Saha*et al.,* 2012).

The colorimetric detection of nucleic acid using GNP is widely used due to the extraordinary importance of nucleotide sequences in biological analysis. It hasas advantages such as simplicity, easy preparation, low cost and time saving (Jazayeri*et al,* 2018). GNPs have been used to detect DNA with high sensitivity and selectivity (Daniel et al., 2004; Sun *et al*., 2009). Mutations, single nucleotide polymorphisms (SNPs), chromosomal translocations, gene expression, and pathogens from clinical samples can be easily detected (Baptista et al., 2005; Doria et al., 2007; Sun *et al*., 2009). Taking the advantage of colorimetric detection, our study was plan to detect *S. aureus* genomic DNA using GNP conjugated probe.

The *nuc* gene code for nuclease enzyme and nucleotide sequence poses apparently unique sequences of the *S. aureus*. So, nuc specific primers are used for PCR based detection of *S. aureus*by many workers (Brakstad*et al*., 1992; Kim *et al*., 2001). Thiol group is most widely used interaction to achieve the stable conjugation between DNA oligonucleotides and GNP (Li *et al.,* 2013).

Though various detection techniques are available to diagnose *S. aureus* but they are limited to research/diagnostic laboratories there is a dearth of knowledge about the biosensing of pathogenic *S. aureus* at the farmer's doorstep, which can sensitivitily diagnose sub-clinical mastitis. In view of above-mentioned fact, the present study was aimed towards evaluation of oligonucleotide probe conjugated GNPfor rapid detection of S. aureus. The study with following objective.

1. **To design an oligonucleotide probe for *S. aureus* detection**
2. **To evaluate the newly designed probe as bioreceptor for the optical biosensing of S. aureus**

2. Review of Literature

There are many procedures for diagnosis of *S. aureus.* One of the common diagnosis methods is use of specific culture media that needs at least numerous days for detection (Abdelhamid *et al*., 2014). In recent years, genetic identification methods, especially using the technique of polymerase chain reaction (PCR) allows significant expansion, quicker identification ability, and more accuracy than the old method (Huang *et al.*, 2006; Martin et al., 2016; Amini *et al* 2017). The analysis of PCR results by electrophoresis are needed for equipment, materials, and a long duration of time because of the presence of PCR inhibitors; in this method, sometimes, the result can be falsely negative. In advanced PCR models, such as real-time PCR, resonant fluorescence energy transmissions are utilized that accelerate the detection process providing probes, however, is still expensive (Shi *et al*., 2015; Mehta *et al.*, 2015; Narmani *et al*., 2018).

Biosensors show new investigative equipment which are sensitive, fast and portable. (Ivnitski*et al*.,1999). Biosensors are devices at the interface of biology with microsystems technology, which combine a biological element (bioreceptor) with a physical transducer, the sensor (Alhadrami, 2018). According to the transducing component, the biosensors can be shifted to five groups, Electrochemical biosensor (voltametric, amperometric, potentiometric, and conductometric), Calorimetric biosensor, Thermometric biosensor, Optical biosensor (colorimetric, luminescent, fluorescent, and interferometric), and Mass-based biosensor (gravimetric/acoustic wave and magnetic or piezoelectric) (Pinto *et al.,* 2012).

The primary biosensor which was based on enzyme electrode projected by Clark in 1962 (Manna *et al*., 2013). These biosensors employ biological sensing elements, like modified transducers, to hold measurement of numerous analytes. (Pablos e*t al*., 2009). Biosensors have a very vast range of applications like food safety, disease detection, drug discovery, environmental monitoring, defence and so on, with the purpose of increasing quality of life (Arora *et al.,* 2011). Nanoparticles have properties such as very small sizes, surface-to-volume ratios, and unique physical and chemical characteristics, which large-scale materials lack. In fact, the size of particle makes it a possibility to overcome the quantum force. This also affects the particle's properties during its reaction to other particles (Chen *et al.,* 2013).

2.1 Gold nanomaterials

Gold nanomaterials with a wide variety of shapes, such as rods, spheres, cubes, wires, and cages, GNP are the most popular nanomaterials for sensor applications (Xiao *et al*., 2020). Their intrinsic properties, such as good biocompatibility, large surface area, ease of synthesis and modification, and high chemical and thermal stability make Au nanomaterial ideal choice as substrates and transducers for sensors. The optical properties of these materials depend on their size and shape which can be controlled during the synthesis process. Besides, the surface of GNP can be modified by electrostatic, hydrophobic, and covalent bonding (Peng *et al.,* 2018).

2.2 Gold nanoparticle (GNP)

GNP (GNP)is one of the most extensively studied nanomaterials. It is polycrystalline gold nanostructure with an apparently spherical shape and typically 5-100 nm diameter size. Typical GNP could be synthesized via two well-known approaches, the chemical reduction and the ligand passivation (Goulet *et al*., 2010; Peng *et al.,* 2018). The chemical reduction method by using tri-sodium citrate for the reduction of gold hydrochloride was first reported by Turkevich *et al* (1951). The Turkevich method is a relatively simple and reproducible technique for the synthesis of spherical particles between 10 nm to 30 nm. However, the particles become less spherical, the size distribution becomes broader, and the results were less reproducible for the synthesis of GNP above 30 nm size. This method has been further modified in order to achieve more precise control over the particle size and size distribution by adjusting the reaction conditions by many workers (Li *et al*., 2011; Schulz *et al.,* 2014). Sodium borohydride and ascorbic acid are strong reducing agents, so they can be used to produce GNP in conjunction to the citrate ions that act as capping ligands.

There are other reducing agents being utilized for GNP preparation with the dual roles of reducing potential and desired stabilizers, including organic molecules, bacteria, microorganisms, plant extracts, liposomes, polymer, and inorganic reagents (Setyawati *et al.,* 2014). Alteration the sizes of GNP could be achieved during the synthesis process by optimizing the reaction condition. For example, Frens (1973) reported that increasing size of GNP could be produced by decreasing the ratio of gold chloride/citrate ions in the reaction system. Piella, et al (2016) controlled the GNP size through stepwise seed mediated route in which the smaller GNP produced in an earlier synthesis step is used as a seed for the subsequent growth of the bigger GNP.

In electrochemical sensors, GNP has found application due to their unique ability to improve the charge transfer processes (Fei *et al*., 2015) and the electrode's conductivity (Güner, *et al.,* 2017). GNP are extensively applied as a colorimetric indicator, because of their surface plasmon resonance property (Khoshbin, *et al*., 2018 and Phanchai*et al*., 2018). Along with their optical and electrochemical properties, GNP are very attractive in biosensing since their high surface area offer unmatched opportunities for high loading of biomolecules such as antibodies, enzymes, and aptamers; moreover, GNP have been shown to maintain high bioactivity of immobilized biomolecules (Fei *et al.,* 2015).

2.3 Biosensors for diagnosis of disease based on nanomaterials

Biosensors are devices at the interface of biology with microsystems technology, which combine a biological element (bioreceptor) with a physical transducer, the sensor (Alhadrami 2018). In general, when the biological recognition element interacts with a target molecule, the interaction creates a measurable signal that can be converted into data by the integrated transducer. There are several types of transducing principles, but the most commonly studied and in use for pathogen detection are electrochemical (Rotariu*et al*., 2016; Zhang *et al*., 2019), optical (Yoo and Lee, 2016), or fiber optic surface plasmon resonance (SPR) (Dudak and Boyaci, 2009) and piezoelectric (mass-based) (Pohanka, 2018). Calorimetric, gravimetrical, magnetic, and acoustic sensors are among other examples (Welbeck *et al*., 2011; Valderrama*et al*., 2016; Umesha and Manukumar, 2018).

Depending on the type of transducer and nature of the target entity, different recognition elements (probes), can be used (Morales and Halpern, 2018). Single stranded oligonucleotides, antibodies or parts thereof, and enzymes are common examples of recognition elements used in sensing bacterial contamination. Less frequent, but emerging as promising alternatives, are bacteriophages, aptamers, peptide nucleic acids (PNAs), and other engineered affinity ligands, such as artificial binding proteins and molecularly imprinted polymers (MIPs) (Vidic*et al.,* 2017).

2.3.1 Electrochemical biosensors (EC)

An electrochemical (EC) biosensor is a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information. EC biosensors are based on the measurement of changes in current, potential, conductance, or field effect due to the interaction of the target molecule with the biorecognition element

(BRE) on the sensing surface. Also, screen printed technology facilitates the largescale fabrication of portable, low-cost analytical systems that can operate with small sample volume (Eissa*et al*., 2018). EC biosensors are preferred due to their robustness, selectivity, sensitivity, analytical performances than other biosensors (Sheikhzadehet*et al.,* 2016 and Housaindokht*et al.,* 2014).

EC biosensors are based on the three-electrode system i.e. working electrode, auxiliary/counter electrode and a reference electrode (Gattani*et al*., 2019, Zhang *et al*., 2017). The fundamental principle behind the working of the EC biosensor lies in the transfer kinetics of ions/electrons from the reaction center to the electrode surface. Both in label-free and labelled EC biosensors, the increase or decrease in electron transfer is measured Also, screen printed technology facilitates the large-scale fabrication of portable, low-cost analytical systems that can operate with small sample volume (Eissa*et al.,* 2018).

2.3.2 Fluorescence biosensors

Fluorescence-based sensors have attracted great interest in presenting high sensitivity, low detection limit, rapid response, low signal-to-noise ratio, simple instrumentation, and cost-effectiveness. A variety of fluorescence nano-materials such as metal nanoparticles, quantum dots (QDs), carbon dots (CDs), graphene oxide, dye-doped silica nanobeads, nanotubes, and up conversion nanoparticle have been explored in sensing application (Bhardwaj *et al.,* 2017). Signal generation in fluorescence sensing has been carried out following a variety of strategies including fluorescence quenching (“turn-off”), fluorescence enhancement (“turn-on”), ratio metric (calculating intensity ratio of two or more fluorescence signal), fluorescence resonance energy transfer (FRET), photoinduced electron transfer (PET) and metal enhanced fluorescence (MEF) (Li *et al*., 2019).

2.3.3 Surface-enhanced Raman scattering (SERS) biosensors

Surface-enhanced Raman scattering (SERS) is a powerful sensing tool; this presents several advantages including high sensitivity, reduced analysis time, portability as well as the ability for multiplex detection. SERS technology takes advantage of the enhancement of the Raman spectroscopy signal when performed at the surface of noble metal nanomaterials; signal enhancements of several orders of magnitude (Hao*et al.,* 2017 and Lu *et al*., 2017) were reported using this method. SERS is a fascinating method for the identification (fingerprinting) and detection of microorganisms because of its non-destructive nature.

Nanomaterials like noble metal colloids, nanospheres, core-shell, gold-coated magnetic nanoparticle, nano aggregates, and bimetallic nanomaterial have been reported as promising materials for SERS detections using both labels (indirect) and label-free (direct) approaches. Labels (SERS reporters) are specific organic molecules like, Rhodamine B, 4-Nitrothiophenol 4-mercaptopurine (4-MPY), 4-amino thiophenol (4-ATP), 4-mercaptobenzoic acid and5,5-dithiobis-2-nitrobenzoic acid (DTNB) that are characterized by well-known Raman signals. Plasmonic nanoparticles decorated with Raman reporter molecules and biorecognition elements have been used to design SERS biosensors (Liu *et al*., 2017).

2.3.4 Lateral flow-based biosensors

Lateral-flow immunoassay (LFIA) also known as lateral-flow immunochromatographic (LF- ICA) tests are simple, rapid, and portable systems which enable qualitative, non-invasive point of care detection (POC) with wider applications in medical diagnostic (Koczula*et al*., 2016). Conventional LFA system suffers from low sensitivity; nevertheless, in recent times several approaches for the improvement of LFA have reported including gold enhancement, enzyme labelling, increased loading of antibody, and use of various nanomaterials like QD, UCNP, peroxidase-like nanoparticle, and graphene oxide. Besides coupling LFIA systems with instrumental reading (fluorescence, colorimetric, SERS, and magnetic focus) enabled to obtain more reliable and quantitative data (Pan *et al*., 2018).

2.3.5 Colorimetric biosensors

Colorimetry is a well-established method with widely usedin sensor development. This transduction method has advantages like low cost, simple/no instrumentation, and ease of fabrication. Naked-eye detection is a fascinating branch of colorimetric methods that enables qualitative detection of analytes; on the other end, the use of spectrophotometric reading allows quantitative measurements (Xu *et al*., 2019). GNP is among the most used material in colorimetric detection. The different detection approached based on Au nanomaterials can be classified into three “groups”: (i) approaches based on aggregation/disaggregation, (ii) approaches based on catalytic properties of the Au nanomaterials, and (iii) growth of hybrid nanomaterials (Peng *et al*., 2018).

GNP exhibit different physical and chemical properties when they reach the nanoscale; there are significant changes in the colour of the nanoparticles that depending on the size of the nanoscale (Fu *et al*., *2016* and Luo *et al*., 2014). The principles of the GNP colorimetric method are that if the genomic DNA is present in the environment, the probes are complemented by them. Then, the addition of salt causes the accumulation of GNP and, as a result, changes the colour of environment. In the absence of genomic DNA, the probes will remain with the GNP in the solution without any reaction; thus, no colour change occurs (Shahbaz *et al.,* 2018).

DNA conjugated with GNP are used in a variety of molecular diagnostic applications such as high sensitivity DNA detection in homogenous solution (Reynolds *et al.,* 2000) and microarray- based DNA detection (Cao *et al*., 2006) lab-on-chip based substrates (Park *et al*., 2002) atomic force microscopy based detection (Bui et al., 2007) detection of polymerase chain reaction (PCR) amplicons (Ozsoz *et al.,* 2003; Cai *et al.,* 2010) The optical property of colloidal gold nano particles forms the basis of its application in rapid and specific hybridization based DNA detections. This property has already been applied for detection of eukaryotic gene expression (Baptista *et al*., 2005), *Mycobacterium tuberculosis* detection (Liandris *et al*., 2009) and for RNA quantification to detect chronic myeloid leukaemia (Conde et al., 2010).

Shahbaz *et al* (2018) reported that colorimetric assay was about three times more sensitive than the PCR method and this assay presents a satisfactorily linear range, low detection limit, good accuracy and specificity in the detection of the *S. aureus* target gene. Similarly, Deb *et al* (2022) detected *E coli* specific DNA in milk samples of cow affected with mastitis in a quick and easy manner using GNP based colorimetric assay. Bakthavathsalam *et al* (2012) demonstrate the colorimetric assay is highly sensitive and reliable screening tool for rapid detection of *Escherichia coli* causing urinary tract infection. The detection of presence of bacteria could be performed with high sensitivity and specificity within the turnaround time of 30 minutes.

3. MATERIALS

3.1 Materials

3.1.1. Chemicals and Reagents

All The chemicals, buffers, media and reagents used in this study were high pure grade obtained from reputed manufacturers viz. Sigma (USA), Fermentas (USA), SRL (India), Merck (India/Germany) and Hi-media (India). Details and composition of different reagents, solutions, media and buffers used in the present study are given in the appendix or at appropriate places. All the chemical solutions were prepared using HPLC grade water.

3.1.2. Bacteria and Bacteriological media:

Staphylococcus aureus, Escherichia coli, Aeromonas hydrophila and *Clostridium perfringens* culture obtained from the department of veterinary public health and epidemiology, Bihar Veterinary College, Patna. Mannitol salt agar used as selective media for *S. aureus.* Nutrient broth was used as growth media for *S. aureus* in this study. All media were sterilized by autoclaving.

3.1.3. Plastic glassware and other consumables

All the glassware were procured from Borosil. All the plastic wares like petri-plates, cell culture plates, centrifuge tubes of various capacities, micropipette tips, microcentrifuge tubes of 0.5 ml, 1.5 ml and 2 ml, thin-walled PCR tubes, syringe filter were procured from different reputed firms. All the plastic wares were certified non-pyrogenic and nuclease free and required plastic were sterilized by autoclaved.

3.1.4. Instruments

Air displacement micropipettes *viz.* P10, P100, P1000 (Coring, USA), Gel electrophoresis apparatus-Horizontal (Tarsons, India) Gel documentation system- (Bio-Rad, USA) Refrigerated microcentrifuge (Beckman Coulter, USA), Refrigerator, Whirlpool, India Ltd, -20^{0}C Deep freezer (Vestfrost, USA), -80^{0}C freezer (New Brunswick Scientific, USA), Nano spectrophotometer (Cole-palmer, US), Spectrophotometer (Systonic, India),Thermal cycler (Bio-Rad, USA), microwave oven (LG), Double distillation apparatus (Borosil,

India),Weighing balance (Mettler and toledo, India).

3.1.5. Probes and Primers:

Primer and probe used in this study are listed in table 1. Primer and 5' thiol modified probe was custom synthesize from Integrated DNA Technologies, India.

Table 1: List of primer and Probe

Primer	Sequence (5'-3')	Remarks
NUCF	5'-GCGATTGATGGTGATACGGTT-3'	Brakstad *et al.,* 1992
NUCR	5'-AGCCAAGCCTTGACGAACTAAAGC-3'	
Probe	5'-TGATTGTCAAACTCGACTTCAA-3'	*Nuc* gene specific

3.2. Methodology

3.2.1. S*taphylococcus aureus*

The culture of *S. aureus* was revived from the department of veterinary public health and epidemiology, Bihar Veterinary College, Patna in 5ml of nutrient broth and incubated overnight at 37°C. Mannitol salt agar plates were streaked with broth cultures to isolate the single colonies and glycerol stock prepared from culture in 30 % glycerol and stored at -80^{0}C. In this study *S. aureus* was grown in Mannitol salt agar or nutrient broth.

3.2.2. Isolation of *Staphylococcus aureus* genomic DNA

The genomic DNA was isolated following the method of Wilson (1987) with modifications. About 0.5 ml of 16 hr broth culture (single colony, inoculated in nutrient broth) was inoculated in 50 ml of nutrient broth and subjected to incubation at 37^{o}C under constant shaking for 16 hrs. The pellet obtained by centrifugation at 3500g for 15 min at 4^{o}C, was washed twice with GTE and then suspended in 3.6 ml buffer. After that 400 µl lysozyme (20mg/ml in GTE buffer) was added and incubated at 37^{o}C for 1 hr. Then 400µl SDS (10% w/v) and 5µl of proteinase K (20 mg/ml) was added to cell suspension and incubated at 37^{o}C for 30 min. The lysate was treated with 1ml of 5M NaCl and 800µl Cetyl Trimethyl Ammonium Bromide (CTAB) (10%w/v in 0.7M NaCl w/v) and further incubation at 65^{o}C in a water bath for 15 min. Equal volumes of the Phenol chloroform mixture (1:1 v/v) was added to each tube, mixed gently and centrifuged at 12,000 X g for 10 min. The supernatant was collected in a separate tube and DNA was precipitated by adding 0.1 volumes of 3 M ammonium acetate (pH5.2) and 0.6 volumes of Isopropanol and kept at room temperature for

2 hrs. DNA was pelleted by centrifugation at 15,000 g for 10 min and pellet was washed with 70% ethanol and air dried, then pellet was dissolved in 100 µl nuclease free water containing RNaseA (20mg/ml), incubated at 37^0C for 1 hr. Stored at -20^oC till further use.

3.2.3. Checking purity and concentration of DNA

The absorbance of extracted DNA was measured at 260nm and at 280 nm in nano spectrophotometer. The ratio of OD_{260} and OD_{280} was calculated to check the purity of the extracted DNA.

3.2.4.PCR amplification of nuc gene of *Staphylococcus aureus*

Amplification of *nuc* gene was carried out by PCR using *nuc* gene primers (Brakstad*et al.*, 1992) which amplify 267 bp product. The PCR reaction was set up in a standard 25 reaction volume using the following reagents (Fermantas) in a 0.2 ml PCR tube on ice.

Component	**Quantity**
10 X PCR buffer (Dream Taq)	2.5 µl
dNTP (10mM)	0.5 µl
Dream Taq DNA Polymerase (5U)	0.2 µl
Forward primer NUCF (10 pmol/µl)	2 µl
Reverse primer NUCF (10 pmol/µl)	2 µl
*Staphylococcus aureus*Genomic DNA	3 µl
Nuclease free water	Up to 25 µl

The cyclic condition used for amplification were as follows

Steps	**Temperature**	**Time**	**Condition**
	Heated lid 99 ^{0}C		
Step I	94ºC	5 min	Initial denaturation
Step II	94ºC	1 min	Denaturation

Step III	60ºC	1 min	Primer annealing
Step IV	72ºC	1 min	Extension
	Step II to Step IV repeated 35 cycles		
Step V	72ºC	10 min	Final Extension

The reaction product was analysed by agarose gel electrophoresis. PCR product was purified by using PCR clean-up system (Promega) and its concentration was measured at 260 nm in nano spectrophotometer. Purified PCR product was also sent for sequencing. Sequence result obtained from IDT was used for designing of probe.

3.2.5. Designing of probes:

Probe sequence was designed for *nuc* gene sequence obtained after sequencing hat has homology among the *S. aureus* strains. A 22 base oligonucleotide probe was designed using IDT primer quest tool Care is taken in designing the sequence so that it does not share any sequence homology with non- *S. aureus* family members. Additionally, the melting temperature of the probes was ensured to be within a narrow range. Further the sequence is checked for potential self-complementarities and also formation of secondary structures was verified using oligoanalyser software which may otherwise hinder the assay. The final 22 base probe sequence obtained was custom synthesize from IDT with 5' end thiol modification.

3.2.6. Chemical synthesis of gold nanoparticle (GNP)

Colloidal GNP was synthesized by using the citrate-reduction method as describe by (Shahbazi *et a*l., 2018) with slight modification. Briefly 20 µl of 50 mM gold chloride solution was dissolved in 50 ml water and boiled on hot plate with stirring. Then 2ml of 1.5 % sodium citrate was added drop by drop till color changed to red. After that solution was allow to boiling for another five min and finally allowed to cool them.

3.2.7. Spectrophotometric Characterization of Gold nano particle

Spectrum analysis of citrate GNP was performed from 400 to 700 nm range using UV-Visible spectrophotometry to know the λ_{max}.

3.2.8. Conjugation of thiolated oligonucleotide probe over gold nanoparticle (GNP)

Conjugation of 5' thiol modified oligonucleotide probe was performed as per Nill and Mirkin, (2006) with slight modifications. Briefly 4 n mol of probe was mixed with 30µl of 10nM TCEP (Annexure-II) and 5µl of 0.5M Sodium acetate buffer (Annexure-II), the mixture incubated for 1 hr (in brown coloured tube or wrapped in aluminium foil) after incubation added 1 ml of GNP (20 nM) citrated and the mixture was incubated in orbital shaker at speed of 120 rpm for 15 hrs. There after 104.5 µl of 100mM, pH- 7.0phosphate adjustment buffer (Annexure-II) and 11.49 µl of 1% SDS was (Annexure-II) added. The mixture was kept in continuous shaking for 30 min at speed of 120 rpm. 29µlof 0.3M NaCl and divided into six equal volumes was added at interval of 8 hrs. The mixture was washed three times with washing buffer (Annexure-II). There after probe GNP mixture was centrifuged at 10000 X g for 20 min at 4°C and supernatant was discarded and pellet was three times washed with 1ml of washing buffer (Annexure-II). Finally, pellet was re-suspended in 50µl washing buffer and stored at 4°C till further study. Spectrum analysis of GNP Probe was performed from 400 to 700 nm range in spectrophotometer to know the shift in band of GNP conjugated with oligonucleotide probe.

3.2.9. Determination of optimal concentration of HCl for the Aggregation of GNP

GNP aggregated in highly acidic medium (Dahl *et al.,* 2020). This experiment was done to identify the minimum concentration of HCl require to aggregate of GNP. Upon aggregation the red colour of GNP would turn into blue. In this experiment, mixture of 4 µl PBS, 1 µl Nuclease free water and 5 µl GNP conjugated probe in 0.2 ml PCR tube was prepared and heated in thermo cycler at 60ºC for 20 min. Different concentration HCl which comprised of 0 mM (control) 80 mM,100 mM,120 mM and 140 mM were prepared by using serial dilution, and they were added to this mixture after incubation at room temperature for 10 min The appearances of colour were recorded and compared. The colour change was observed at 140 mM HCl concentration. Again, this experiment was performed with 120 mM ,130 mM and 140 mM HCl concentration and at 130 mM of HCl colour change was observed. 130 mM of HCl was used in hybridization experiment.

3.2.10. GNP oligonucleotide probe hybridization assay

This experiment was performed as per method described by Bakthavathsalam *et al.,* (2012). Briefly a complementary oligonucleotide (5' ACTGGAAACAGTTTGAGCTGAAGTT 3')

specific to the probe sequence was obtained from Eurofins genomics. 1µl of 100 p mol complementary oligonucleotide in 4 µl PBS was heated to 95°C for 5 min. Then 5 µl GNP oligo probe was added immediately followed by incubation at 60°C for 20 minutes. This experiment was performed in duplicate. After hybridization the one sample was subjected to acid challenge and other subjected to β-mercaptoethanol treatment to remove GNP from hybridised GNP Probe. The colour of the solution was noted five minutes after the addition of 130 mM HCl and photographed.

The β-mercaptoethanol treatment was performed as per method of Li, *et al*., (2013). This was accomplished by mixing 1.3 M β-mercaptoethanol 5µl with GNP hybridised probe and kept for 24 hrs at room temperature in incubator. Then the mixture is centrifuged for 5 min and supernatant is collected in another tube was allowed to migrate in 2% agarose gel at 100V for1hr. Hybridised oligo was visualised with ethidium bromide.

3.2.11. Hybridization assay with PCR amplicon of *nuc* gene

The hybridization assay was performed similar to GNP oligonucleotide probe hybridization assay except that PCR amplicon of nuc gene was used instead of complementary oligonucleotide. Tenfold dilution of purified PCR product (3.49 µg/ ml) till 6 dilutions i.e., 0, 10, 10^2, 10^3, 10^4, $10^5$was prepared. 1µl of diluted PCR product was mixed with 4µl of buffer in a PCR tube. The mixture was heated at 95°C for 5 min in thermo cycler to denature the DNA template. After denaturation,5µl of GNP oligo probe was added and kept immediately at 60°C for 20 min to allow hybridization after hybridization the hybridized product was kept at room temperature for 10 min. After hybridization each tube were treated with 130 mM HCl and change in colour was observed.

3.2.12. Hybridization assay with *S. aureus* genomic DNA

The hybridization assay with genomic DNA was performed in the vein of PCR amplicon hybridization assay. Only difference is that genomic DNA isolated from *S. aureus* was used in hybridization assay. Tenfold dilution of genomic DNA (12.59 µg/ ml) till 6 dilutions i.e. 0, 10, 10^2, 10^3, 10^4, 10^5 was prepared. 1µl of genomic DNA was mixed with 4µl of buffer in a PCR tube. The mixture was heated at 95°C for 5 min in thermo cycler to denature the DNA template. After denaturation, 5µl of GNP oligo probe was added and kept immediately at 60°C for 20 min to allow hybridization after hybridization the hybridized

product was kept at room temperature for 10 min. After hybridization each tube were treated with 130 mM HCl and change in colour was observed.

3.2.13. Specificity of the assay with different bacterial genomic DNA

To determine the specificity of the assay, DNA from three different organisms (*Escherichia coli, Aeromonas hydrophila* and *Clostridium perfringens*) obtained from department of veterinary public health and epidemiology, Bihar veterinary College, Patna were extracted and subjected to hybridization assay. 1µl of genomic DNA isolated from different bacteria was mixed with 4µl of buffer in a PCR tube. The mixture was heated at 95°C for 5 min in thermo cycler to denature the DNA template. After denaturation 5µl of GNP oligo probe was added and kept immediately at 60°C for 20 min to allow hybridization after hybridization the hybridized product was kept at room temperature for 10 min. After hybridization each tube were treated with 130 mM HCl and change in colour was observed.

4. Result

4.1. Isolation of *Staphylococcus aureus* genomic DNA

DNA extracted from the culture of *Staphylococcus aureus* was 100 times diluted and it had A_{260}/A_{280} ratio of 1.85 and the concentration of DNA was 12.59 µg/ml. This genomic DNA was used as template for PCR amplification of *nuc* gene and hybridization experiment with GNP probe.

4.2. Amplification of *nuc* gene

Nuc gene of *S. aureus* was amplified by PCR using nuc specific primers. The PCR gave amplified product of 267 bp (Fig.1). The concentration and A_{260}/A_{280} ratio of DNA was 3.49 µg/ml. and 1.82 respectively. Sequencing result of obtained from vendor show similarity with *S. aureus* genome sequence upon NCBI-BLAST. This PCR product was also used for hybridization experiment with GNP probe.

4.3. Probe designing

Probe was designed from sequence obtained from sequencing of *nuc* gene using IDT primer quest tool. The sequence of probe was 5'-TGATTGTCAAACTCGACTTCAA -3'. When probe sequence were BLAST on NCBI probe shows similarity with *S. aureus* genome sequence. Probe do not form any secondary structure when analysed with oligoanalyser software.

4.4. Chemical synthesis of gold nanoparticle (GNP)

Colloidal GNP was synthesized by citrate-reduction method and colour was red (Fig.2.) GNP was conjugated with 5' thiol modified oligonucleotide probe. Spectrum analysis of shows shift in λ_{max} of GNP 515.2 nm to 521 nm upon conjugation with thiol modified oligonucleotide probe (Fig.3).

4.5. Determination of optimal concentration of HCl for the aggregation of GNP

The normal colour GNP probe mixture was red and addition of certain amount HCl change the colour of GNP probe due to aggregation of GNP. Colour change was observed in GNP probe at 130 mM concentration of HCl (Fig.4) upon treatment with different

concentrations of HCl at 20 mM increment from 80 mM to 140 mM. Again, this experiment was performed with 120 mM ,130 mM and 140 mM HCl concentrations and at 130 mM of HCl colour change was observed. This concentration of HCl was used in all hybridization experiment for observation of colour change.

4.6. GNP oligonucleotide probe hybridization assay

Initially, the GNP oligo probe assay was optimized for visual detection using 22 base complementary sequences. It was found that optimum temperature for hybridization is 60°C. The complementary DNA after hybridization with the GNP probe was treated with 130 mM HCl and colour was change to purple from red (fig.5). Gel electrophoresis was performed after β-mercaptoethanol treatment below 100 bp was observed (Fig.6).

4.7. Hybridization assay with PCR amplicon of *nuc* gene

The hybridization assay was applied to PCR amplicon of *nuc* gene. The concentration PCR product of *nuc* gene3.49 µg/ml. Result in fig. 7 shows no change in colour up to 5^{th} dilution. Colour change was observed from 6^{th} dilution. The concentration of DNA at 5^{th} dilution was 0.35 ng/ml.

4.8. Hybridization assay with *S. aureus* genomic DNA

Hybridization assay was also performed with S. aureus genomic DNA. The concentration of genomic DNA was 12.59 µg/ml. No change in colour was observed up to 5^{th} dilution upon treatment with 130 mM HCl. The colour change was observed from 6^{th} dilution as shown in fig. 8. The concentration of genomic DNA at 5^{th} dilution was 1.26 ng/ml.

4.9. Specificity of the assay can be detected by different bacterial genomic DNA

To determine the specificity of the assay, hybridization assay was performed with genomic DNA of three different bacteria i.e., *Escherichia coli, Aeromonas hydrophila* and *Clostridium perfringens.* Upon addition of 130 mM HCl genomic DNA *E. coli, A. hydrophila* and *C. perfringens* shows change in colour from red to purple while genomic DNA of *S. aureus*do not show any change in colour (fig. 9).

5. Discussion

Bovine mastitis a major disease affecting dairy cattle worldwide and it results from the inflammation of the mammary gland. On the basis of the inflammation severity, mastitis can be classified into sub-clinical, clinical and chronic forms. Sub-clinical mastitis is difficult to detect due to the absence of any visible indications, and it has major cost implications.

In this study we target *Staphylococcus aureus* because it is one of the most significant contagious pathogens (Liapi *et al*., 2021). There are many procedures for diagnosis of *S. aureus*. One of the common diagnosis methods is use of specific culture media that needs at least numerous days for detection (Abdelhamid *et al.,* 2014). These are reasonable and sensitive, but this method is time consuming (Huang *et al.,* 2006). Techniques based on nucleic acid methods for example, polymerase chain reaction (PCR) have high compassion and high specificity for diagnosis of *S. aureus*, but these methods need expert technicians and require complicated procedures (Shi *et al.,* 2015).

The colorimetric detection of nucleic acid using gold nanoparticles (GNP) is widely used technique due to the extraordinary importance of nucleotide sequences in biological analysis as well as the characteristics and advantages of using GNP in detection, such as simplicity, easy preparation, low cost and time saving (Jazayeri *et al*, 2018). GNPs have been used to detect DNA with high sensitivity and selectivity (Daniel et al., 2004; Sun *et al*., 2009). Mutations, single nucleotide polymorphisms (SNPs), chromosomal translocations, gene expression, and pathogens from clinical samples can be easily detected (Baptista et al., 2005; Doria et al., 2007; Sun *et al*., 2009). Taking the advantage of colorimetric detection, our study was planned to detect *S. aureus* genomic DNA using GNP conjugated probe.

The *nuc* gene specific probe was designing because the nucleotide sequence of *nuc* gene poses apparently unique sequences of the *S. aureus*. Designed probe shows similarity only with *S. aureus*, it indicates that probe is specific to *S. aureus* only. Thiol group is most widely used interaction to achieve the stable conjugation between DNA oligonucleotides and GNP (Li *et al.,* 2013). So, 5’ thiol modified probe was used for conjugation with GNP.

GNP synthesis via the reduction of gold chloride by citric acid is most widely used method (Polte *et al.,* 2010). GNPs synthesized by citrate reduction of gold chloride are stable in solution, duo to electrostatic repulsion among citrate ion-coated GNP which prevents them from aggregation. In general, GNP with mean diameters of 5–20 nm has a visible λ_{max} around 520 nm caused by the surface plasmon band (Schrekker *et al.,* 2007) In our study the visible λ_{max} at 515.2 nm for GNP, which indicated the formation of GNP.

Successful conjugation of probes to the GNP is must for the entire diagnostic process. Shift in λ_{max} from 515.2 nm to 521 nm indicate successful conjugation of probes to GNP. Increase in λ_{max} was also observed by Peng *et al* (2015) and Shahbazi *et al* (2018).

Colour change from red to purple was obsorbed by decrease in pH upon addition of 130 mM HCl to conjugated GNP. While below 130 mM HCl concentration no colour change was obsorbed. It indicates that 130 mM HCl is optimal concentration for GNP aggregation. Previous research demonstrated that single stranded DNA (ssDNA) probe has a protective effect on GNP against aggregation due to highly charged phosphate backbone of ssDNA sustains the electrostatic repulsion among ssDNA conjugated GNP (Yang *et al*., 2006). Addition of excess of H^+ ion may neutralize the negative charges of phosphate groups of DNA, leading to GNP' aggregation and colour change (Sun *et al*., 2009).

Initially, the GNP oligo probe assay was optimized for visual detection using 22 base complementary DNA sequences. It was found that optimum temperature for hybridization is 60°C for GNP probe and the target DNA. Colour change was observed from red to purple when hybridised GNP oligo with complementary DNA due to aggregation of GNP. Hybridization of GNP oligo probe with the target DNA formed double stranded DNA helix that stabilizes the GNP under acidic environment and hence remains red. However, in the absence of complementary DNA the GNP oligo probes aggregate under acidic environment and the solution turns purple (Bakthavathsalam et al., 2012). The complementary DNA after hybridization with the GNP probe was treated with β-mercaptoethanol and separated on gel electrophoresis followed by visualised with ethidium bromide. Since ethidium bromide can bind to double stranded DNA, appearance of band in gel confirms hybridization of GNP probe with target DNA.

When hybridization assay was performed with PCR amplicon of *nuc* gene, no visible change in colour of the solution occurred upto sample containing 0.35 ng/ml of PCR amplicon of *nuc* gene below which the solution turned purple indicating the aggregation of

the GNP oligo probes. Thus GNP oligo probe was found to detect the presence of 0.35 ng/ml of PCR product of target pathogen after acid induced aggregation defining the minimum detection limit of the assay.

To determine the sensitivity of this colorimetric method various dilution of *S. aureus* genomic DNA was also used to determine the limit of detection of the GNP oligo probe assay with genomic DNA. It was observed that no visual colour shift from red to purple in solution up to sample containing 1.26 ng/ml of genomic DNA. It shows that 1.26 ng/ml was minimum detection limit for this assay. Similar kind of colour change was also observed by Bakthavathsalam *et al* (2012) with Escherichia coli genomic DNA and by Shahbazi *et al* (2018) with *S. aureus* genomic DNA.

In order to determine the specificity, The GNP oligo probe assay was applied to the genomic DNA isolated from *S. aureus, Escherichia coli, Aeromonas hydrophila* and *Clostridium perfringens* It was observed that the solution containing *S. aureus*, genomic DNA remains red, whereas the solution containing DNA of other bacteria turned purple after addition of HCl. Thus, this assay can differentiate *S. aureus* and non *S. aureus* genomic DNA by necked eye without using any sophisticated instrument.

6. Summary and conclusion

India is the largest producer of milk in the world and it accounts for 22% of the world milk production. Mastitis is the most significant disease of dairy herds. Subclinical mastitis (SCM) in cows is more prevalent in India than clinical mastitis. Mastitis is a multi etiologic multifaceted disease, which is distinct as inflammation of parenchyma of mammary glands and is considered by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissues. The mastistis causing bacterial species are *Escherichia Coli, Staphylococcus aureus, Streptococcus agalactiae, and Corynebacterium bovis* etc. *S. aureus* causes one of the most common forms of chronic mastitis. The conventional and PCR based method for identification of pathogen is time consuming, most cases require highly specialized personnel, dedicated equipment. There is need to develop less lengthy approaches, capable of providing on-site information concerning the infection. Optical biosensors for rapid detection of pathogens are steadily gaining popularity due to its simplicity and sensitivity. Gold nanoparticles (AuNPs) possess distinct physical and chemical properties that make them excellent scaffolds for the fabrication of novel chemical and biological sensors. Surface plasmon resonance (SPR) providing unique optical properties to gold nano-particle.

This study was initiated by isolating the genomic DNA from *S. aureus* was revived from the department of veterinary public health and epidemiology, Bihar Veterinary College. There after amplification of *nuc* gene was carried out by PCR using *nuc* gene, which amplified 267 bp product. PCR product was purified and sent for sequencing. The *nuc* gene specific probe was designed because the nucleotide sequence of *nuc* gene poses apparently unique sequences of the *S. aureus*. Designed probe shows similarity only with *S. aureus*, it indicates that probe is specific to *S. aureus* only. Thiol group is most widely used interaction to achieve the stable conjugation between DNA oligonucleotides and GNP. So, 5' thiol modified probe was used for conjugation with GNP.

GNP synthesis via the reduction of gold chloride by citric acid had visible λ_{max} at 515.2 nm for GNP, which indicated the formation of GNP. Shift in λ_{max} from 515.2 nm to 521 nm indicate successful conjugation of probes to GNP. Colour change from red to purple was absorbed by decrease in pH upon addition of 130 mM HCl to conjugated GNP. While below 130 mM HCl concentration no colour change was absorbed. It indicates that 130 mM HCl is optimal concentration for GNP aggregation.

Initially, the GNP oligo probe assay was optimized for visual detection using 22 base complementary DNA sequences. It was found that optimum temperature for hybridization is 60°C for GNP probe and the target DNA. Colour change was observed from red to purple when hybridised GNP oligo with complementary DNA due to aggregation of GNP. Hybridization of GNP oligo probe with the target DNA formed double stranded DNA helix that stabilizes the GNP under acidic environment and hence remains red. However, in the absence of complementary DNA the GNP oligo probes aggregate under acidic environment and the solution turns purple. The complementary DNA after hybridization with the GNP probe was treated with β-mercaptoethanol and separated on gel electrophoresis followed by visualised with ethidium bromide. Since ethidium bromide can bind to double stranded DNA, appearance of band in gel confirms hybridization of GNP probe with target DNA.

When hybridization assay was performed with PCR amplicon of *nuc* gene, no visible change in colour of the solution occurred up to sample containing 0.35 ng/ml of PCR amplicon of *nuc* gene below which the solution turned purple indicating the aggregation of the GNP oligo probes. Thus, GNP oligo probe was found to detect the presence of 0.35 ng/ml of PCR product of target pathogen after acid induced aggregation defining the minimum detection limit of the assay. To determine the sensitivity of this colorimetric method various dilution of *S. aureus* genomic DNA was also used to determine the limit of detection of the GNP oligo probe assay with genomic DNA. It was observed that no visual colour shift from red to purple in solution up to sample containing 1.26 ng/ml of genomic DNA. It shows that 1.26 ng/ml is detection limit for this assay. In order to determine the specificity, The GNP oligo probe assay is applied to the genomic DNA isolated from *S. aureus*, *Escherichia coli*, *Aeromonas hydrophila* and *Clostridium perfringens* It is observed that the solution containing *S. aureus*, genomic DNA remains red, whereas the solution containing DNA of other bacteria turned purple after addition of HCl. Thus, this assay can differentiate *S. aureus* and non *S. aureus* genomic DNA by necked eye without using any sophisticated instrument.

To conclude this method is rapid and convenient, and can be accomplished within 30-40 min and a significant colour change that is easily recognized by the naked eye. This assay presents a satisfactorily linear range, low detection limit, good accuracy and specificity in the detection of the *S. aureus* target DNA. Furthermore, our method is suitable for detection of detection of *S. aureus* genomic DNA from clinical sample and it can be applied as a promising candidate for the on-site detection of S. aureus.

7. Bibliography

Abdelhamid H. N., Khan M. S., and Wu H. F. (2014). Design, characterization and applications of new ionic liquid matrices for multifunctional analysis of biomolecules: A novel strategy fo**r** pathogenic bacteria biosensing. *Analyticachimicaacta*, ***823***, 51-60.

Albrecht M.A., Evans. C.W. and Raston. C.L. 2006. Green chemistry and the health implicationsofnanoparticles.*Green Chemistry*, **8**, 417–432.

Alhadrami H. A. (2018). Biosensors: Classifications, medical applications, and future prospective. *Biotechnology and applied biochemistry*, ***65*(3)**, 497-508.

Amini B., Kamali M., Salouti M., and Yaghmaei P. (2017). Fluorescence bio-barcode DNA assay based on gold and magnetic nanoparticles for detection of Exotoxin A gene sequence. *Biosensors and Bioelectronics*, *92*, 679-686.

Anderson K. L., Lyman R., Moury K., Ray D., Watson D. W., and Correa M. T. (2012). Molecular epidemiology of Staphylococcus aureus mastitis in dairy heifers. *Journal of dairy science*, ***95*(9)**, 4921-4930.

Arora P., Sindhu A., Dilbaghi N., and Chaudhury A. (2011). Biosensors as innovative tools for the detection of food borne pathogens. *Biosensors and Bioelectronics*, ***28*(1)**, 1-12.

Bakthavathsalam P., Rajendran V. K., and Baquir Mohammed J. A. (2012). A direct detection of Escherichia coli genomic DNA using gold nanoprobes. *Journal of nanobiotechnology*, **10(1),** 1-10.

Baptista P., Doria G., Henriques D., Pereira E., and Franco R. (2005). Colorimetric detection of eukaryotic gene expression with DNA-derivatized gold nanoparticles. *Journal of biotechnology*, **119(2),** 111-117.

Bhardwaj N., Bhardwaj S. K., Nayak M. K., Mehta J., Kim K. H., and Deep A. (2017). Fluorescent nanobiosensors for the targeted detection of foodborne bacteria. *TrAC Trends in Analytical Chemistry*, ***97***,120-135.

Brakstad O. G., Aasbakk K., and Maeland J. A. (1992). Detection of Staphylococcus aureus by polymerase chain reaction amplification of the nuc gene. *Journal of clinical microbiology*, ***30*(7)**, 1654-1660.

Bui M. P. N., Baek T. J., and Seong G. H. (2007). Gold nanoparticle aggregation-based highly sensitive DNA detection using atomic force microscopy. *Analytical and bioanalytical chemistry*, ***388*****(5),** 1185-1190.

Cai M., Li F., Zhang Y. And Wang Q. (2010). One-pot polymerase chain reaction with gold nanoparticles for rapid and ultrasensitive DNA detection. *Nano Research*, ***3*****(8),** 557-563.

Cao X., Wang Y. F., Zhang C. F., and Gao W. J. (2006). Visual DNA microarrays for simultaneous detection of Ureaplasma urealyticum and Chlamydia trachomatis coupled with multiplex asymmetrical PCR. *Biosensors and Bioelectronics*, *22*(3), 393-398.

Cesewski E., and Johnson B. N. (2020). Electrochemical biosensors for pathogen detection. *Biosensors and Bioelectronics*, ***159***, 112214.

Chen L., Lu, W., Wang X., and Chen L. (2013). A highly selective and sensitive colorimetric sensor for iodide detection based on anti-aggregation of gold nanoparticles. *Sensors and Actuators B: Chemical*, ***182***, 482-488.

Cheng M. S., Ho J. S., Tan C. H., Wong J. P. S., Ng L. C., and Toh C. S. (2012). Development of an electrochemical membrane-based nanobiosensor for ultrasensitive detection of dengue virus. *Analyticachimicaacta*, ***725***, 74-80.

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

Conde J., de la Fuente J. M., and Baptista P. V. (2010). RNA quantification using gold nanoprobes-application to cancer diagnostics. *Journal of Nanobiotechnology*, ***8*****(1),** 1-8.

Dahl G. T., Krueger J. D., Döring S., Weller H., and Vossmeyer T. (2020). Seeded growth synthesis of Zirconia@ Gold particles in aqueous solution. *Nanomaterials*, ***10*****(6)**, 1197.

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

Deb R., Pal, P., Chaudhary P., Bhadsavle S., Behera M., Gautam D., Roshan M., Vats A., Ludri A., Gupta V.K. and De S. and De S. (2022). Development of gold nanoparticle-

based visual assay for rapid detection of Escherichia coli specific DNA in milk of cows affected with mastitis. *LWT*, **155,** 112901.

Dell'Atti D., Zavaglia M., Tombelli S., Bertacca G., Cavazzana A. O., Bevilacqua G., Minunni M and Mascini M. (2007). Development of combined DNA-based piezoelectric biosensors for the simultaneous detection and genotyping of high risk Human Papilloma Virus strains. *Clinica chimica acta*, ***383*(1-2)**, 140-146.

Demers L. M., Östblom M., Zhang H., Jang N. H., Liedberg B., and Mirkin C. A. (2002). Thermal desorption behavior and binding properties of DNA bases and nucleosides on gold. *Journal of the American Chemical Society*, ***124*(38)**, 11248-11249.

Dideikin A. T., and Vul A. Y. (2019). Graphene oxide and derivatives: the place in graphene family. *Frontiers in Physics*, ***6***, 149.

Ding S. Y., You E. M., Tian Z. Q., and Moskovits M. (2017). Electromagnetic theories of surface-enhanced Raman spectroscopy. *Chemical Society Reviews*, ***46*(13)**, 4042-4076.

Doria G., Franco R., and Baptista P. (2007). Nanodiagnostics: fast colorimetric method for single nucleotide polymorphism/mutation detection. *IetNanobiotechnology*, **1(4),** 53-57.

Du K., Cai H., Park M., Wall T. A., Stott M. A., Alfson K. J., Griffiths A., Carrion R., Patterson J.L., Hawkins A.R. and Mathies R. A. (2017). Multiplexed efficient on-chip sample preparation and sensitive amplification-free detection of Ebola virus. *Biosensors and Bioelectronics*, ***91***, 489-496.

Dudak F. C., and Boyacı İ. H. (2009). Rapid and label-free bacteria detection by surface plasmon resonance (SPR) biosensors. *Biotechnology Journal: Healthcare Nutrition Technology*, ***4*(7)**,1003-1011.

Eissa S., Alshehri N., Rahman A. M. A., Dasouki M., Abu-Salah K. M., and Zourob M. (2018). Electrochemical immunosensors for the detection of survival motor neuron (SMN) protein using different carbon nanomaterials-modified electrodes. *Biosensors and Bioelectronics*, ***101***,282-289.

Elsheshtawy A., Yehia N., Elkemary M., and Soliman H. (2019). Direct detection of unamplified Aeromonashydrophila DNA in clinical fish samples using gold nanoparticle probe-based assay. Aquaculture, **500**, 451-457.

Farokhzad O.C and Langer R. (2009). Impact of nanotechnology on drug delivery.*ACSNanotechnology*,**3**,16–20.

Farooq U., Yang Q., Ullah M. W., and Wang S. (2018). Bacterial biosensing: Recent advances in phage-based bioassays and biosensors. *Biosensors and Bioelectronics*, ***118***, 204-216.

Faye B. and Konuspayeva G. 2012. The sustainability challenge to the dairy sector– The growing importance of non-cattle milk production worldwide. International Dairy Journal, **24 (2)**: 50-56.

Fei J., Dou W., and Zhao G. (2015). A sandwich electrochemical immunoassay for Salmonella pullorum and Salmonella gallinarum based on a AuNPs/SiO_2/Fe_3O_4 adsorbing antibody and 4 channel screen printed carbon electrode electrodeposited gold nanoparticles. *RSC Advances*, ***5*(91)**,74548-74556.

Fracchiolla N. S., Artuso S., and Cortelezzi A. (2013). Biosensors in clinical practice: focus on oncohematology. *Sensors*, ***13*(5)**, 6423-6447.

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

Fu X., Cheng Z., Yu J., Choo P., Chen L., and Choo J. (2016). A SERS-based lateral flow assay biosensor for highly sensitive detection of HIV-1 DNA. *Biosensors and Bioelectronics*, ***78***, 530-537.

Gattani A., Singh S. V., Agrawal A., Khan M. H., and Singh P. (2019). Recent progress in electrochemical biosensors as point of care diagnostics in livestock health. *Analytical Biochemistry*, **579**, 25-34.

Goulet P. J., and Lennox R. B. (2010). New insights into Brust− Schiffrin metal nanoparticle synthesis. *Journal of the American Chemical Society*, *132*(28), 9582-9584.

Griesche C., and Baeumner A. J. (2020). Biosensors to support sustainable agriculture and food safety. *TrAC Trends in Analytical Chemistry*, ***128***, 115906.

Guliy O. I., Zaitsev B. D., Larionova O. S., and Borodina I. A. (2019). Virus detection methods and biosensor technologies. *Biophysics*, ***64*(6)**, 890-897.

Güner A., Çevik E., Şenel M., and Alpsoy L. (2017). An electrochemical immunosensor for sensitive detection of Escherichia coli O157: H7 by using chitosan, MWCNT,

polypyrrole with gold nanoparticles hybrid sensing platform. *Food chemistry*, ***229***, 358- 365.

Hao L., Gu H., Duan N., Wu S., Ma X., Xia Y., Tao Z. and Wang Z. (2017). An enhanced chemiluminescence resonance energy transfer aptasensor based on rolling circle amplification and WS2 nanosheet for Staphylococcus aureus detection. *Analyticachimicaacta*, ***959***,83-90.

Hill H. D., and Mirkin C. A. (2006). The bio-barcode assay for the detection of protein and nucleic acid targets using DTT-induced ligand exchange. *Nature protocols*, ***1*****(1)**, 324-336.

Housaindokht M. R., Ashraf N., and Sheikhzadeh E. (2014). Design of a disposable solid state potentiometric sensor for codeine content control in pharmaceutical preparations. *Sensor Letters*, ***12*****(9)**,1341-1346.

Hu X., Dou W., and Zhao G. (2015). Electrochemical immunosensor for Enterobactersakazakii detection based on electrochemically reduced graphene oxide–gold nanoparticle/ionic liquid mo https://vikaspedia.in/agriculture/livestock/role-of-livestock- in-indian-dified electrode. *Journal of Electroanalytical Chemistry*, ***756***, 43-48.

Hu Y., Li, H., and Li J. (2018). A novel electrochemical biosensor for HIV-related DNA detection based on toehold strand displacement reaction and cruciform DNA crystal. *Journal of Electroanalytical Chemistry*, ***822***, 66-72.

Huang H., Bai W., Dong C., Guo R., and Liu Z. (2015). An ultrasensitive electrochemical DNA biosensor based on graphene/Au nanorod/polythionine for human papillomavirus DNA detection. *Biosensors and Bioelectronics*, ***68***, 442-446.

Huang S. H. (2006). Gold nanoparticle-based immunochromatographic test for identification of Staphylococcus aureus from clinical specimens. *Clinicachimicaacta*, ***373*****(1-2)**, 139-143.

Ivnitski D., Abdel-Hamid I., Atanasov P., and Wilkins E. (1999). Biosensors for detection of pathogenic bacteria. *Biosensors and Bioelectronics*, ***14*****(7)**, 599-624.

Jazayeri M. H., Aghaie T., Avan A., Vatankhah A., and Ghaffari, M. R. S. (2018). Colorimetric detection based on gold nano particles (GNPs): An easy, fast,

inexpensive, low-cost and short time method in detection of analytes (protein, DNA, and ion). *Sensing and bio-sensing research*, ***20,*** 1-8.

Khoshbin Z., Housaindokht M. R., Verdian A., and Bozorgmehr M. R. (2018). Simultaneous detection and determination of mercury (II) and lead (II) ions through the achievement of novel functional nucleic acid-based biosensors. *Biosensors and Bioelectronics*, ***116***, 130- 147.

Kim C. H., Khan M., Morin D. E., Hurley W. L., Tripathy D. N., Kehrli Jr, M., Oluoch A. O. and Kakoma I. (2001). Optimization of the PCR for detection of Staphylococcus aureus nuc gene in bovine milk. *Journal of dairy science*, *84*(1), 74-83.

Koczula K. M., and Gallotta A. (2016). Lateral flow assays. *Essays in biochemistry*, ***60*****(1)**, 111-120.

Konishi Y., Ohno K., Saitoh N., Nomura T and Nagamine S. 2008. Microbial synthesis of goldnanoparticles by metal reducing bacterium.*Journal of Biochemical and BiophysicalMethods*,**29**,2341-2343.

Koskinen M. T., Wellenberg G. J., Sampimon O. C., Holopainen J., Rothkamp A., Salmikivi L., Van Haeringen W.A., Lam T.J.G.M. and Pyörälä S. (2010). Field comparison of real- time polymerase chain reaction and bacterial culture for identification of bovine mastitis bacteria. Journal of Dairy Science, **93(12)**, 5707-5715.

Laurence J. C. (2005). Hepatitis A and B immunizations of individuals infected with human immunodeficiency virus. *The American journal of medicine*, ***118*****(10)**, 75-83.

Lengke M andSoutham G. 2006. Bioaccumulation of gold by sulfate-reducing bacteriaculturedin the presence of gold(I)-thiosulfate complex.*Geochimica et CosmochimicaActa* ,**70**,3646–3661.

Li C., Li, D., Wan G., Xu J., and Hou W. (2011). Facile synthesis of concentrated gold nanoparticles with low size-distribution in water: temperature and pH controls. *Nanoscale research letters*, *6*(1), 1-10.

Li Y., Wang Z., Sun L., Liu L., Xu C., and Kuang H. (2019). Nanoparticle-based sensors for food contaminants. *TrAC Trends in Analytical Chemistry*, ***113***, 74-83.

Li F., Zhang H., Dever, B., Li X. F., and Le X. C. (2013). Thermal stability of DNA functionalized gold nanoparticles. Bioconjugate chemistry, **24(11),** 1790-1797.

Liandris E., Gazouli M., Andreadou M., Čomor M., Abazovic N., Sechi L. A., and Ikonomopoulos J. (2009). Direct detection of unamplified DNA from pathogenic mycobacteria using DNA-derivatized gold nanoparticles. *Journal of microbiological methods*, ***78*****(3),** 260-264.

Liapi M., Botsaris G., Arsenoglou C., Markantonis N., Michael C., Antoniou A., and Pipis C. (2021). Rapid Detection of Mycoplasma bovis, Staphylococcus aureus and Streptococcus agalactiae in Cattle Bulk Tank Milk in Cyprus and Relations with Somatic Cell Counts. *Pathogens*, **10(7)**, 841.

Liu Y., Zhou H., Hu Z., Yu G., Yang D., and Zhao J. (2017). Label and label-free based surface-enhanced Raman scattering for pathogen bacteria detection: A review. *Biosensors and Bioelectronics*, ***94***,131-140.

Lu D., Pang G., and Xie J. (2017). A new phosphothreoninelyase electrochemical immunosensor for detecting Salmonella based on horseradish peroxidase/GNPs-thionine/chitosan. *Biomedical microdevices*, ***19*****(1)**, 12.

Luo R., Li Y., Lin X., Dong F., Zhang W., Yan L., Cheng W., Ju H. and Ding S. (2014). A colorimetric assay method for invA gene of Salmonella using DNAzyme probe self-assembled gold nanoparticles as single tag. *Sensors and Actuators B: Chemical*, ***198***, 87- 93.

Manna S. K., Maurye P., Dutta C., and Samanta G. (2013). Occurrence and virulence characteristics of aeromonas species in meat, milk and fish in I ndia. *Journal of Food Safety*, ***33*****(4)**, 461-469.

Martins S. A., Martins V. C., Cardoso F. A., Germano J., Rodrigues M., Duarte C., Bexiga, R., Cardoso S. and Freitas P. P. (2019). Biosensors for on-farm diagnosis of mastitis. Frontiers in bioengineering and biotechnology, **7,** 186.

Martin J. G. P., e Silva G. D. O., da Fonseca C. R., Morales C. B., Silva C. S. P., Miquelluti, D. L., and Porto E. (2016). Efficiency of a cleaning protocol for the removal of enterotoxigenic Staphylococcus aureus strains in dairy plants. *International Journal of Food Microbiology*, ***238*****,** 295-301.

Mehta M. S., McClure J. T., Mangold K., and Peterson L. R. (2015). Performance of 3 real-time PCR assays for direct detection of Staphylococcus aureus and MRSA from clinical samples. *Diagnostic microbiology and infectious disease*, ***83*****(3**), 211-215.

Metkar S. K., and Girigoswami K. (2019). Diagnostic biosensors in medicine–a review. *Biocatalysis and agricultural biotechnology*, ***17***, 271-283.

Mirkin C. A., Letsinger R L., Mucic R. C. and Storhoff J. J. (1996). A DNA-based method for rationally assembling nanoparticles into macroscopic materials. *Nature*, **382**(6592), 607-609.

Morales M. A., and Halpern J. M. (2018). Guide to selecting a biorecognition element for biosensors. *Bioconjugate chemistry*, ***29*****(10)**, 3231-3239.

Narayanan K.B and Sakthivel N. (2010). Biological synthesis of metal nanoparticles bymicrobes.*Advances in Colloids and Interface Science,***156**,150-158.

Narmani A., Kamali M., Amini B., Kooshki H., Amini A., and Hasani L. (2018). Highly sensitive and accurate detection of Vibrio cholera O1 OmpW gene by fluorescence DNA biosensor based on gold and magnetic nanoparticles. *Process Biochemistry*, ***65,*** 46-54.

Ozsoz M., Erdem A., Kerman K., Ozkan D., Tugrul B., Topcuoglu N., Ekren H. and Taylan M. (2003). Electrochemical genosensor based on colloidal gold nanoparticles for the detection of Factor V Leiden mutation using disposable pencil graphite electrodes. *Analytical chemistry*, ***75*****(9),** 2181-2187.

P. R. (2019). Epidemiological studies of mastitis in cows reared under different managemental system in and around Parbhani. *The Pharma Innovation Journal*, ***8*****(2)**, 01 05.

Pablos M., Rodríguez-Calleja J. M., Santos J. A., Otero A., and García-López M. L. (2009). Occurrence of motile Aeromonas in municipal drinking water and distribution of genes encoding virulence factors. *International journal of food microbiology*, ***135*****(2)**, 158-164.

Pan R., Jiang Y., Sun, L., Wang R., Zhuang K., Zhao Y., Wang H., Ali M.A., Xu H. and Man C. (2018). Gold nanoparticle-based enhanced lateral flow immunoassay for detection of Cronobacter sakazakii in powdered infant formula. *Journal of dairy science*, ***101*****(5)**, 3835-3843.

Pandey A., Gurbuz Y., Ozguz, V., Niazi J. H., and Qureshi A. (2017). Graphene-interfaced electrical biosensor for label-free and sensitive detection of foodborne pathogenic E. coli O157: H7. *Biosensors and Bioelectronics*, ***91***, 225-231.

Park S. J., Taton T. A., and Mirkin C. A. (2002). Array-based electrical detection of DNA with nanoparticle probes. *Science*, *295*(5559), 1503-1506.

Peng L., Li B. L., Zhou C. W., Li N. B., Setyawati M. I., and Zou H. L. (2018). "Naked-eye" recognition: Emerging gold nano-family for visual sensing. *Applied Materials Today*, ***11***, 166-188.

Peng H., Wang C., Xu X., Yu C., and Wang Q. (2015). An intestinal Trojan horse for gene delivery. *Nanoscale*, *7*(10), 4354-4360.

Phanchai W., Srikulwong U., Chompoosor A., Sakonsinsiri C., and Puangmali T. (2018). Insight into the molecular mechanisms of AuNP-based aptasensor for colorimetric detection: a molecular dynamics approach. *Langmuir*, ***34*(21)**, 6161-6169.

Philip D. 2009. Biosynthesis of Au, Ag and Au–Ag nanoparticles using edible mushroomextract.*Spectrochimicaacta* ,**33**,374–381.

Piella J., Bastus N. G., and Puntes V. (2016). Size-Controlled Synthesis of Sub-10-nanometer Citrate-Stabilized Gold Nanoparticles and Related Optical Properties. *Chemistry of Materials*, ***28*(4),** 1066-1075.

Pinto A. D., Terio V., Pinto P. D., and Tantillo G. (2012). Detection of potentially pathogenic Aeromonasisolatesfrom ready-to-eat seafood products byPCR analysis.*International journal of food science* and *technology*, ***47*(2)**,269-273.

Pohanka M. (2018). Overview of piezoelectric biosensors, immunosensors and DNA sensors and their applications. *Materials*, ***11*(3)**, 448.

Polte J., Ahner T. T., Delissen F., Sokolov S., Emmerling F., Thünemann A. F., and Kraehnert R. (2010). Mechanism of gold nanoparticle formation in the classical citrate synthesis method derived from coupled in situ XANES and SAXS evaluation. *Journal of the American Chemical Society*, *132*(4), 1296-1301.

Pratim B and Chang-Yu W. (2005). Nanoparticles and the environment.*Journal of the Air* and *Waste Management Association*,**55**,708–746.

Quintela I. A., de Los Reyes B. G., Lin C. S., and Wu V. C. (2019). Simultaneous colorimetric detection of a variety of Salmonella spp. in food and environmental samples by optical biosensing using oligonucleotide-gold nanoparticles. *Frontiers in microbiology*, ***10***, 1138.

Rai M., Gade, A., Gaikwad S., Marcato P. D., and Durán N. (2012). Biomedical applications of nanobiosensors: the state-of-the-art. *Journal of the Brazilian Chemical Society*, ***23*(1)**, 14-24.

Reynolds R. A., Mirkin C. A., and Letsinger R. L. (2000). Homogeneous, nanoparticle-based quantitative colorimetric detection of oligonucleotides. *Journal of the American Chemical Society*, *122*(15), 3795-3796.

Riddin T.L., Gericke M and Whiteley C. 2006. Analysisof the intra and extracellular formationof platinum nanoparticles byFusariumoxysporumf.sp. lycopersici using surface responsemethodology.*Nanotechnology*,**14,**3482-3489.

Rotariu L., Lagarde F., Jaffrezic-Renault N., and Bala C. (2016). Electrochemical biosensors for fast detection of food contaminants–trends and perspective. *TrAC Trends in Analytical Chemistry*, ***79***,80-87.

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

Schrekker H. S., Gelesky M. A., Stracke M. P., Schrekker C. M., Machado G., Teixeira S. R., Rubim J.C. and Dupont J., and Dupont J. (2007). Disclosure of the imidazolium cation coordination and stabilization mode in ionic liquid stabilized gold (0) nanoparticles. *Journal of Colloid and Interface Science*, ***316*(1),** 189-195.

Schulz F., Homolka T., Bastús N. G., Puntes V., Weller H., and Vossmeyer T. (2014). Little adjustments significantly improve the Turkevich synthesis of gold nanoparticles. Langmuir, 30(35), 10779-10784.

Setyawati M. I., Xie J., and Leong D. T. (2014). Phage based green chemistry for gold ion reduction and gold retrieval. *ACS Applied Materials* and *Interfaces*, *6*(2), 910-917.

Shahbazi R., Salouti M., Amini B., Jalilvand A., Naderlou E., Amini A., and Shams A. (2018). Highly selective and sensitive detection of Staphylococcus aureus with gold nanoparticle-based core-shell nano biosensor. *Molecular and cellular probes*, ***41***, 8-13.

Shaikh S. R., Digraskar S. U., Siddiqui M. F., Borikar S. T., Rajurkar S. R., and Suryawanshi P. R. (2019). Epidemiological studies of mastitis in cows reared under different managemental system in and around Parbhani. *The Pharma Innovation Journal*, ***8*(2)**, 01-05.

Sharma H., Maiti S. K. and Sharma K. K. (2007). Prevalence, etiology and antibiogram of microorganisms associated with subclinical mastitis in buffaloes in drug, Chhattisgarh state. Int. J. Dairy Sci. **2**:145-151.

Sharma N., Maiti S.K. and Koley K.M. (2004). Studies on the incidence of sub clinical mastitis in buffaloes of Rajnandgaon district of Chhattisgarh state. Vet. Prac. **5**: 123-124

Sharma A., Sharma N., Kumari A., Lee H. J., Kim T., and Tripathi K. M. (2020). Nano-carbon based sensors for bacterial detection and discrimination in clinical diagnosis: A junction between material science and biology. *Applied Materials Today*, ***18***, 100467.

Sheikhzadeh E., CHamsaz M., Turner A. P. F., Jager E. W. H., and Beni V. (2016). Label-free impedimetric biosensor for Salmonella Typhimurium detection based on poly [pyrrole-co-3-carboxyl-pyrrole] copolymer supported aptamer. *Biosensors and bioelectronics*, ***80***, 194-200.

Shi J., Chan C., Pang Y., Ye W., Tian F., Lyu J., Zhang Y. and Yang M. (2015). A fluorescence resonance energy transfer (FRET) biosensor based on graphene quantum dots (GQDs) and gold nanoparticles (AuNPs) for the detection of mecA gene sequence of Staphylococcus aureus. *Biosensors and Bioelectronics*, ***67***,595-600.

Shiying H., Zhirui G., Zhanga Y., Zhanga S., Wanga J and Ning G. (2006). Biosynthesis of goldnanoparticles using the bacteriaRhodopseudomonascapsulata.*Material Letter*,**61**,84-91.

Singh A., Choudhary M., Singh M. P., Verma H. N., Singh S. P., and Arora K. (2015). DNA functionalized direct electro-deposited gold nanoaggregates for efficient detection of Salmonella typhi. *Bioelectrochemistry*, ***105***, 7-15.

Slawson R.M., Van M.I., Lee H and Trevor J.T. (1992). Germanium and silverresistance,accumulation and toxicity in microorganisms.*Plasmid* ,**27**,73-79.

Solaimuthu A., Vijayan A. N., Murali P., and Korrapati P. S. (2020). Nano-biosensors and their relevance in tissue engineering. *Current Opinion in Biomedical Engineering*, ***13***, 84-93.

Stringer R. C., Schommer S., Hoehn D., and Grant S. A. (2008). Development of an optical biosensor using gold nanoparticles and quantum dots for the detection of porcine

reproductive and respiratory syndrome virus. *Sensors and Actuators B: Chemical*, ***134*(2)**, 427-431.

Sun L., Zhang Z., Wang S., Zhang J., Li H., Ren L., Weng J. and Zhang Q. (2009). Effect of pH on the interaction of gold nanoparticles with DNA and application in the detection of human p53 gene mutation. *Nanoscale research letters*, ***4*(3)**, 216-220.

Sweeney R.Y., Mao C., Gao X., Burt L., Belcher A.M and Georgiou G. 2004. Bacterialbiosynthesis of cadmium sulfidenanocrystals. *Chemical Biology* ,**11**,1553-1559.

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

Umesha S., and Manukumar H. M. (2018). Advanced molecular diagnostic techniques for detection of food-borne pathogens: Current applications and future challenges. *Critical reviews in food science and nutrition*, ***58*(1)**, 84-104.

Valderrama W. B., Dudley E. G., Doores S., and Cutter C. N. (2016). Commercially available rapid methods for detection of selected food-borne pathogens. *Critical reviews in food science and nutrition*, ***56*(9)**,1519-1531.

Viguier C., Arora S., Gilmartin N., Welbeck K., and O'Kennedy R. (2009). Mastitis detection: current trends and future perspectives. Trends in biotechnology, **27(8)**, 486-493.

Vijian D., Chinni S. V., Yin, L. S., Lertanantawong B., and Surareungchai W. (2016). Non-protein coding RNA-based genosensor with quantum dots as electrochemical labels for attomolar detection of multiple pathogens. *Biosensors and Bioelectronics*, ***77***, 805-811.

Welbeck K., Leonard P., Gilmartin N., Byrne B., Viguier C., Arora S., and O'Kennedy R. (2011). Generation of an anti-NAGase single chain antibody and its application in a biosensor-based assay for the detection of NAGase in milk. *Journal of immunological methods*, ***364*(1-2)**, 14-20.

Xiang C., Li R., Adhikari B., She Z., Li Y., and Kraatz H. B. (2015). Sensitive electrochemical detection of Salmonella with chitosan–gold nanoparticles composite film. *Talanta*, ***140***,122-127.

Xiao T., Huang J., Wang D., Meng T., and Yang X. (2020). Au and Au-Based nanomaterials: Synthesis and recent progress in electrochemical sensor applications. *Talanta*, ***206***, 120210.

Xu N., Li J., Gao Y., Zhou N., Ma Q., Wu M., Zhang Y., Sun X., Xie J., Shen G. and Yang. (2019). Apoptotic cell-mimicking gold nanocages loaded with LXR agonist for attenuating the progression of murine systemic lupus erythematosus. *Biomaterials*, ***197***, 380-392.

Yang J., Lee J. Y., Too H. P., Chow G. M., and Gan L. M. (2006). Single stranded DNA stabilization and assembly of Au nanoparticles of different sizes. *Chemical physics*, ***323*****(2-3),** 304-312.

Yoo, S. M., and Lee, S. Y. (2016). Optical biosensors for the detection of pathogenic microorganisms. *Trends in biotechnology*, ***34*****(1)**, 7-25.

Zhang X., Jiang Y., Huang C., Shen J., Dong X., Chen G., and Zhang W. (2017). Functionalized nanocomposites with the optimal graphene oxide/Au ratio for amplified immunoassay of E. coli to estimate quality deterioration in dairy product. *Biosensors and Bioelectronics*, ***89***, 913-918.

Zhang Z., Zhou J., and Du X. (2019). Electrochemical biosensors for detection of foodborne pathogens. *Micromachines*, ***10*****(4)**, 222.

Zhang H., Li, F., Dever B., Li X. F., and Le X. C. (2013). DNA-mediated homogeneous binding assays for nucleic acids and proteins. *Chemical Reviews*, ***113*****(4)**, 2812-2841.

Zhao V. X. T., Wong T. I., Zheng X. T., Tan Y. N., and Zhou X. (2020). Colorimetric biosensors for point-of-care virus detections. *Materials science for energy technologies*, ***3***, 237-249.

Zhu F., Zhao G., and Dou W. (2018). A non-enzymatic electrochemical immunoassay for quantitative detection of *Escherichia coli* O157: H7 using Au@ Pt and graphene. *Analytical biochemistry*, ***559***,34-43.

Zhu H., Zhao G., Wang S. Q., and Dou W. (2018). Photometric sandwich immunoassay for Salmonella pullorum and Salmonella gallinarum using horseradish peroxidase and magnetic silica nanoparticles. *MicrochimicaActa*, ***184*****(6)**, 1873-1880.

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