A novel diagnostic technique for diagnosis of *Staphylococcus aureus* subclinical mastitis using gold nanoparticle-based ELISA

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ABSTRACT

Background: Bovine subclinical mastitis (SCM) is a significant cause of economic losses in dairy farms, primarily due to its asymptomatic nature and difficulty in early detection. The enzyme-linked immunosorbent assay (ELISA) is a widely used diagnostic tool in bioscience, facilitating antigen detection through antibody binding.
Aim: The present study designed a gold nanoparticle (AuNPs)-based ELISA, to enhance the sensitivity of conventional ELISA by improving the binding efficiency of capture antibodies to purified antigens. This modified ELISA could enable more accurate detection of bovine SCM.
Methods: A total of 200 milk samples from apparently healthy cows were screened for SCM using the California Mastitis Test (CMT). Positive samples were then subjected to bacteriological culture, biochemical testing, and polymerase chain reaction targeting the Nuc2 gene for confirmation of *S. aureus*. The purified fraction antigen of all 78 confirmed *S. aureus* isolates was extracted using sepharose 4B affinity column chromatography, described by SDS-polyacrylamide gel electrophoresis, and assessed for its sensitivity in *S. aureus* mastitis diagnosis compared with crude antigen and purified fraction antigen-conjugated gold nanoparticles within an indirect ELISA. Immunoglobulins (IGs) from positive cow serum were extracted and purified from all confirmed *S. aureus*. Gold nanoparticle-based indirect ELISA was used on 400 samples (200 milk and 200 serum) from the same cows.
Results: Using CMT, out of 200 examined milk samples from apparently normal cows, 65% (130/200) were sub-clinically infected. Out of these 130 positive milk samples, 60% (78/130) were confirmed to be infected with *S. aureus*. Purified fraction antigen-conjugated gold nanoparticles achieved the highest sensitivity to ELISA at 97%, 94%, and 92% immediately before storage, 6 months at −20°C, and 1 year at −20°C, respectively. Gold nanoparticle-based indirect ELISA detected specific IgG antibodies in 97% (76/78) sera and milk samples related to confirmed *S. aureus* isolates.
Conclusion: The utilization of purified fraction antigen with gold nanoparticles enhances the sensitivity of ELISA, increasing it from 83.3% to 97% (*p* < 0.01; CI: 99%). The current study establishes a valuable way for *S. aureus* mastitis diagnosis within the use of purified fraction antigen-conjugated gold nanoparticles instead of the classical way to improve the sensitivity and specificity of ELISA.
Keywords: Indirect ELISA, Gold nanoparticle, Subclinical mastitis, *S. aureus*, Antigen purification.

Introduction

Staphylococcus aureus is the major economically significant cause of bovine mastitis that affects dairy cattle globally (Gad *et al.*, 2025). It poses a substantial challenge to the dairy industry, and animal welfare, and a threat to public health. A wide range of virulence genes, such as several toxins, tissue invasion, adhesions, evading the immunity defense mechanism, and evading proteins that enable bacterial colonization, are present in *S. aureus*, allowing it to cause severe and lasting intramammary infections (Fursova *et al.*, 2020).

The disease is characterized by an elevation in somatic cell count, inflammatory cytokines, production of antibodies, and different types of bacteria counts. When *S. aureus* infects the mammary glands, the immune system responds with different types of lymphocytes, as well as leucocytes and cytokines. *Staphylococcus aureus* virulent arsenal allows it to confuse and inhibit the immune system; enterotoxins and toxic shock syndrome toxins primarily cause the pathogenicity of *S. aureus* (Alluwaimi *et al.*, 2003). The primary method for diagnosing mastitis is the quantification of milk inflammatory cells, including macrophages,

lymphocytes, neutrophils, and eosinophils. The California Mastitis Test (CMT) appears to be beneficial as a screening test for the disease (Roshan *et al.*, 2022). Mastitis has a negative economic impact since it reduces milk production, increases cow culling, raises treatment costs, and increases cow death and replacement costs (Al Emon *et al.*, 2024).

Accurate identification and determination of *S. aureus* virulence genes in milk samples is essential for understanding the development of bovine mastitis and designing efficient management measures. ELISA is a commonly used technique for recognizing and quantifying particular proteins or antigens in biological specimens (Sharma *et al.*, 2023). A recent study focused on the application of nanomaterials, including nanogold particles, to ELISA-based assays in an effort to increase sensitivity, and specificity and enhance detection limits (Tabatabaei *et al.*, 2021).

Currently, nanomaterial-improved ELISA is more prevalent because the alteration has greater potential for fast on-time detection as it requires less reagent and can be kept at room temperature for a longer period of time (Juronen *et al.*, 2018). Nanomaterials improve the detection limit of traditional ELISA assays by providing additional binding sites for detection antibodies and improving the signal intensity of this assay (Tabatabaei *et al.*, 2021). The nano-ELISA kit achieved a sensitivity and specificity of 93.33%. This significant advancement in the sensitivity and specificity of the nano-ELISA approach is due to the increased entry of more antibodies into the antigen-antibody complex, resulting in better staining (Khodadadi *et al.*, 2020).

Gold nanoparticles are an effective biomarker for protein and deoxyribonucleic acid analysis because of their extra-ordinary absorption and optic refraction at specific wavelengths, fluorescence characteristics unique to optical detection techniques, high surface-to-volume ratio, and other particular characteristics (Gad *et al.*, 2025). Gold nanoparticles can facilitate the rapid binding of biomolecules such as DNA, antibodies, enzymes, and others, thereby enhancing the number of biochemical detection signals (Khodadadi *et al.*, 2020). Diagnostic systems based on gold nanoparticles (AuNPs) are gaining considerable interest due to their unique surface and extensive absorbance at 520 nanometers (nm) (Parolo *et al.*, 2013). ELISA-based gold nanoparticles typically represent more than one enzyme for each antibody and produce a superior colorimetric signal, making it possible to detect samples with a small analytical number (Gad *et al.* 2025).

Polymerase chain reaction (PCR) is an automated, rapid technique for detecting the most predominant mammary pathogens directly from milk (Hiitiö *et al.*, 2015). Enhancing the diagnostics for subclinical bovine mastitis is crucial to facilitate precise and timely identification of *S. aureus*. In addition, this reduces financial losses and protects public health by preventing mastitis and improving dairy animal

care (Mostafa Abdalhamed *et al.*, 2022). Therefore, this study aims to evaluate a novel approach using gold nanoparticle-based ELISA for the sensitive and specific diagnosis of *S. aureus* in bovine subclinical mastitis (SCM), offering a promising advancement in dairy disease diagnostics.

Materials and Methods

Samples
a) Milk samples: A total of 200 pooled quarter milk samples were aseptically collected from apparent healthy 200 dairy cows on different farms in Egypt between 2022 and 2023. All samples were screened for SCM using the CMT. Of these, 130 milk samples tested positive for SCM and were promptly refrigerated at 4°C for subsequent bacteriological examination.
b) Serum samples: A total of 200 blood samples were collected only from the same cows that prevised the milk samples. Sera were separated and stored at −20°C until subjected to ELISA.

Bacteriological examination
Positive SCM milk samples were streaked onto mannitol salt agar plates (Oxoid, UK) and then incubated at 37°C for 24–48 hours. A spherical convex golden yellow colony was sub-cultured on the same selective media plates and placed in an incubator for 48 hours, at a temperature of 37°C. Following the process of purification, the colonies were recognized morphologically and biochemically, according to Wehr and Frank (2004). Suspected positive *S. aureus* colonies were kept in Brain Heart Infusion (BHI) media containing 50% glycerol for further analysis (Rahim *et al.*, 2021). The confirmation of the suspected colonies was achieved via conventional PCR for *nuc2* gene recognition.

DNA extraction
The purified colonies were extracted by the GF-1 Bacterial DNA Extraction Kit (Cat.no. GF-BA-100, Vivantis, Malaysia) in line with the company protocol. The nucleic acid was extracted using 50 μl of elution buffer. Extracted DNA was observed at 1.5% agarose gel electrophoresis.

Molecular identification using PCR (nuc2) gene
The PCR targeting *S. aureus* was carried out using a GS-96 gradient thermocycler (Hercuvan, Malaysia). A total volume of 25 μl was used, containing 12.5 μl of Master Mix (Cat. W1020300X, Willofort Co., UK), 0.75 μL (10 μm) of each primer, 2 μl of DNA and 9 μl of deionized distilled water. Table 1 reports the cycling conditions and specific primer (*nuc2*) used to detect *S. aureus* according to Abd El-Razik *et al.* (2023).

Preparation and purification of antigen
Pure colonies that were confirmed with PCR were cultured in BHI broth medium, incubated in a shaker incubator at 37°C for 24 hours, and centrifuged at 3,000 rpm for 20 minutes. The supernatant was removed, and the precipitate was washed three times with distilled water. The suspension of bacterial strains was diluted to

Table 1. Cycling conditions and primer sequence of PCR for *NUC2* gene identification.

Gene	Sequence (5′-3′)	Amplicon size (bp)	Cycling conditions
NUC2	AAAGCGATTGATGGTGATACGGTT	664 bp	Init.Denat. 95°C, 10 minutes
	TGCTTTGTTTCAGGTGTATCAACCA		Denat. 95°C, 10 seconds
			Anneal. 56°C, 20 seconds
			Extention. 60°C, 40 seconds
			Final ext. 72°C, 10 minutes
			Cycles. 40

a concentration of 4×10^6 CFU/ml. The crude antigen was prepared through bacterial homogenization in 0.1M pH 7.2 phosphate-buffered saline, according to Al-Mayah and Saeed (2013).

The fraction of *S. aureus* antigen was purified by sepharose 4B affinity column chromatography and then stored for one year at −20°C. The protein of both the crude and purified fraction was measured using a colorimetric test with folin substance following the method described by Lowry *et al.* (1951).

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of proteins
The crude antigen and purified fraction were separately mixed with distinct 2-mercaptoethanol sample buffers. Following the electrophoresis process using 10% reducing polyacrylamide gel, the proteins underwent staining with silver nitrate (Merck, Germany), and the molecular weights of the bands were determined using a protein marker (Sigma-Aldrich) (Laemmli, 1970). Bio Rad Gel Doc XR+ Apparatus was used to determine the molecular weights.

Precipitation and affinity chromatography purification of immunoglobulins (IGs)
IGs from positive cow serum were separated using 50% saturated ammonium sulphate solution, extracted by dialysis for a duration of 3 days (4 times per day at 4°C) using a NaHCO$_3$ buffer (0.1 M, pH 8.3) supplemented with NaCl (0.5 M) and NaN$_3$ (0.02%), and concentrated through lyophilization, following the Abdel-Rahman *et al.* (2017) precipitation process. IGs were purified using protein A Sepharose gel using 0.1 M glycine as an eluting buffer. The purification technique was done according to Abdel-Rahman *et al.* (2017).

Enzyme-linked immunosorbent assay
Sera samples related to PCR confirmed *S. aureus* isolates were subjected to indirect ELISA to assess the sensitivity, and validity of the purified fraction. The concentration of antigens, dilution of antibodies, and dilution of anti-bovine horse radish peroxidase (Sigma) was assessed using checker board titration. The assay was conducted using the crude and purified fraction antigen in accordance with Nakane and Kawaoi (1974). In brief, the microtitration plate was coated with 100 µl of each antigen separately and incubated overnight at 4°C. After washing the plate was blocked with 05% Bovine Serum Albumin in phosphate-buffered saline for 1 hour at room temperature. 100 µl of each serum sample was added to each well after washing. 1.5 hours of incubation at 37°C, the plate was washed and 100 µl of diluted peroxidase conjugated anti-bovine antibodies was added to each well, and the plate was incubated for 1 hour at 37°C. Ortho-Phenlene diamine substrate buffer containing H$_2$O$_2$ was added and the plate was analyzed using spectrophotometry at 450 nm wavelength using the Microplate reader ELx 800 USA.

Design of gold nanoparticle (AuNPs) based ELISA
A total of 200 quarter milk and sera samples (negative and positive *S. aureus*) were subjected to indirect ELISA to assess the sensitivity, and validity of the purified fraction. Milk samples were allowed to defrost at 4°C so that fat could be separated from milk by gravity. A sample of 100 µl was taken from under the fat layer and diluted in 900 µl of sample diluent on a 96-well plate. The technique was similar to above mentioned conventional ELISA using fraction antigen conjugated gold nanoparticles in the conjugation step. In this stage, Gold (III) chloride trihydrate (HAuCl$_4$) solutions were prepared at 1 mM concentrations and diluted in 1 mM 4-morpholinoethanesulfonic acid (MES) buffer at pH 6.0. The AuNPs were formed using H$_2$O$_2$− (hydrogen peroxide) as the reducing agent. The concentration of H$_2$O$_2$− was 100 µM and it was diluted in 1 mM MES buffer at pH 6.0. Based on the published works of Basso *et al.* (2024) Finally, the samples were analyzed using an ELISA reader at a wavelength of 450 nm.

Statistical analysis
The obtained data was analyzed by chi-square using the SPSS for Windows (Version 15.0, USA) statistical software program, and probability (*p*-values) of less than 0.01 was considered significant. Also, sensitivity, specificity, positive predictive value, and negative predictive value were analyzed according to Martin *et al.* (1987).

Ethical approval
The Medical Research Ethics Committee approved this study (No. 13010121) at the National Research Centre, Egypt.

Results

Out of the examined 200 milk samples collected from apparently normal cow, 65% (130/200) showed SCM via the CMT.

Bacteriological examination

Out of 130 positive SCM milk samples, 60% (78/130) showed spherical convex golden yellow colonies and all 60% were confirmed as *S. aureus* via biochemical test. Fifty-two negative *S. aureus* were subjected to indirect gold Nanoparticle (AuNPs) based ELISA.

Molecular identification using PCR

The PCR confirmed *S. aureus* in 100% (78/78) of the bacteriologically positive samples as illustrated in Figure 1.

SDS-PAGE profile of crude and purified fraction antigen

Confirmed *S. aureus* crude and fraction antigen described by SDS–PAGE in Figures 2 and 3 and Table 2. The crude antigen revealed ten bands at molecular weights 245.0, 180.0, 135.0, 100.0, 75.0, 63.0, 48.0, 35.0, 25.0, and 20.0 KDa. The diagnostic fraction was characterized by two bands at 73.1 KDa and 63.9 KDa.

Indirect ELISA

Seventy-eight sera samples related to PCR confirmed *S. aureus* isolates subjected to indirect ELISA to evaluate a crude, fraction, and fraction antigen conjugated gold. The OD reading at 450 nm showed that 83.3% (76/78) of the wells coated with crude antigen had *S. aureus*-specific IgG antibodies, while they increased to 91% (71/78) when coated with fraction antigen and to 97% (76/78) when coated with fraction antigen conjugated with gold as shown in Table 3. Validity evaluation of the stored purified fraction antigen for one year at −20°C compared with freshly purified fraction is revealed in Table 4.

Gold nanoparticle-based indirect ELISA was applied on all 200 milk and serum samples to detect *S. aureus* specific IgG antibodies. Specific IgG antibodies were detected in 97% (76/78) sera and milk samples related to PCR and bacteriologically confirmed *S. aureus* isolates. IgG antibodies were found in 5.7% (3/52) positive SCM and negative *S. aureus* isolate bacteriologically and with PCR. IgG was not detected in any of the seventy negative SCM samples, neither in milk nor in serum.

Superiority intention was applied to treat analysis, the AuNP-based ELISA was compared to standard bacteriological and PCR methods to determine its diagnostic accuracy. Gold nanoparticle-based ELISA has superiority in sensitivity and specificity over traditional methods, which revealed sensitivity, specificity, positive predictive value, and negative predictive value at rates of 97.4%, 97.5%, 96.2%, and 98.3%, respectively.

Discussion

SCM is an asymptomatic form of intra-mammary inflammation that reduces milk production, quality, and overall herd health, causing severe economic losses to the dairy industry, especially that caused by *S. aureus* (Gad *et al.*, 2025). Great attention has recently been given to improving the analysis of mastitis, particularly in the early subclinical stage (Gad *et al.*, 2025).

Somatic cell count estimation is one of the strategies used for the detection of SCM, either directly using automatic somatic cell counters or indirectly by gel formation because the nuclear material of somatic cells excreted in milk works in combination with a specific compound reagent (the mode of action of the CMT) (Elhaig and Selim, 2015).
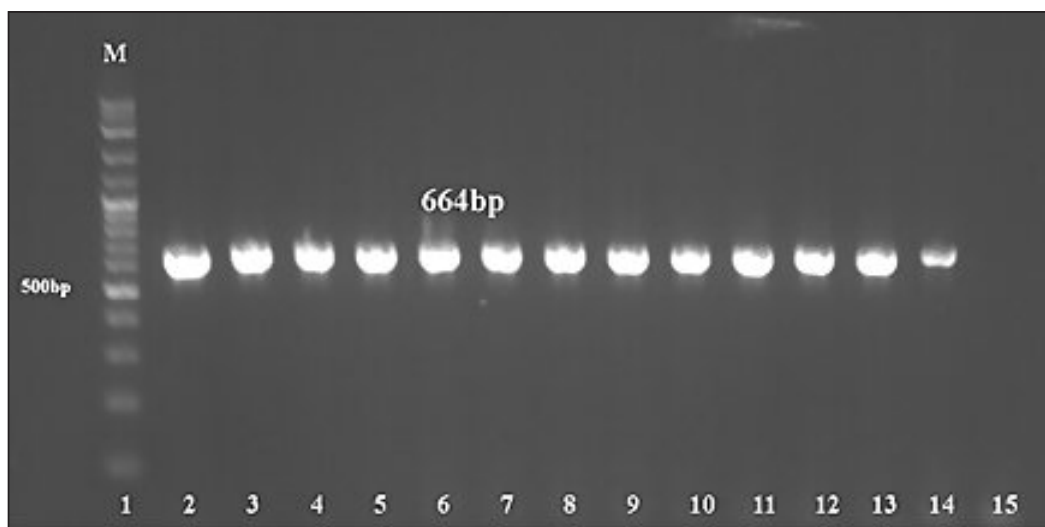

Fig. 1. PCR of *S. aureus nuc2* gene (664 bp). Lane 1 is a 100-bp DNA marker; Lanes 2–13 are representative positive samples. Lane 14: control positive; Lane 15: control negative.
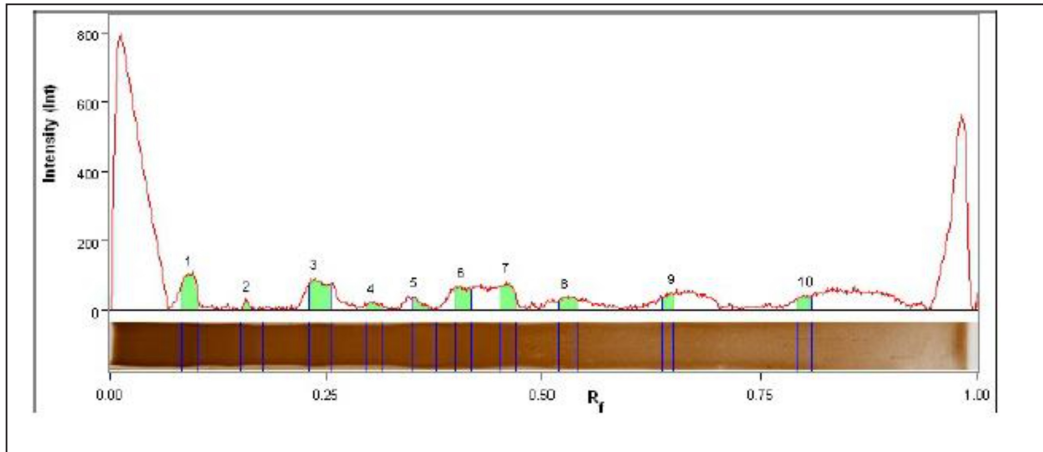
Fig. 2. *Staphylococcal aureus* crude antigen SDS PAGE profile showed 10 bands (10 peaks).

Table 2. SDS PAGE profile of *S. aureus* crude and purified fraction antigen.

	Band No.	Band label	Mol. Wt. (KDa)	Relative front	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
Crude antigen	1	kDa	245.0	0.093	159,100	N/A	N/A	19.6	2.8
	2	kDa	180.0	0.159	22,274	N/A	N/A	2.7	0.4
	3	kDa	135.0	0.237	178,880	N/A	NA	22.0	3.1
	4	kDa	100.0	0.304	33,024	N/A	N/A	4.1	0.6
	5	kDa	75.0	0.352	37,668	N/A	NA	4.6	0.7
	6	kDa	63.0	0.406	98,556	N/A	N/A	12.1	1.7
	7	kDa	48.0	0.459	112,402	N/A	N/A	13.8	19
	8	kDa	35.0	0.524	66,650	N/A	WA	8.2	1.2
	9	kDa	25.0	0.647	48.676	N/A	N/A	6.0	0.8
	10	kDa	20.0	0.803	55,384	N/A	N/A	6.8	1.0
Purified fraction	1	kDa	73.1	0.360	380.920	N/A	N/A	93.3	40
	2	kDa	63.9	0.403	27.499	N/A	N/A	6.7	0.3

Table 3. Indirect ELISA against crude, purified fraction and fraction antigen conjugated gold nanoparticles.

No. of positive sera	positive ELISA against crude antigen	positive ELISA against purified fraction antigen	positive ELISA against fraction antigen conjugated AuNPs
78	83.3% (65/78) [a]	91% (71/78) [a]b	97% (76/78) b

Statistically significant at *p* < 0.01.
(a,b): Columns with different letters are statistically significant.

In the current study, the CMT revealed 65% (130/200) SCM prevalence in different farms in Egypt. This is higher than the prevalence percentage of SCM revealed in other reports conducted in Egypt (52.1%), which were informed by Algammal *et al.* (2020). Differences in geographic distribution, immunological station, unsanitary surroundings, and contaminated apparatuses can all afford elevated rates of SCM in dairy farms, which could be responsible for the elevated rate of *S. aureus* prevalence in the current report.

Among 130 positive SCM milk samples, the prevalence of *S. aureus* in mastitis milk samples using bacteriological examination and biochemical tests was 60% (78/130), lower than that of another study accomplished in Egypt by Abd El-Razik *et al.* (2023) who reported a *S. aureus* prevalence rate of (66.7%)

Table 4. Evaluation of sensitivity and validity of crude, purified fraction and fraction antigen conjugated gold nanoparticles.

	At once	Storage 6 months	Storage 1 year
	Sensitivity	Sensitivity	Sensitivity
Crude antigen	83.3%	81%	78%
Purified fraction antigen	91%	87%	87%
fraction antigen conjugated AuNPs	97%	94%	92%
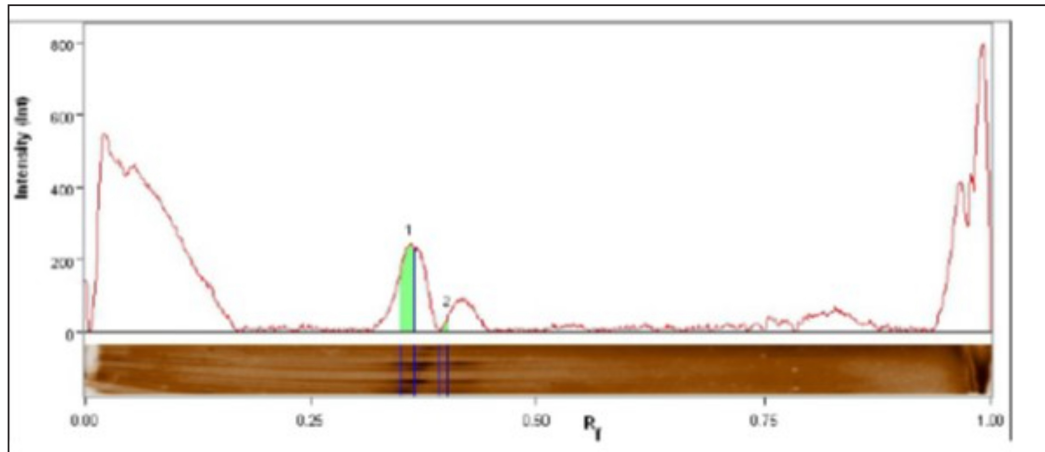
Fig. 3. *Staphylococcal aureus* purified fraction SDS PAGE profile showed 2 bands (2 peaks).

and higher than that of another study achieved in Egypt by Ibrahim *et al.* (2022) who reported a *S. aureus* prevalence rate of 28.92%.

Rapid detection of *S. aureus* is potential throughout the use of molecular techniques like PCR and real time PCR, which are important for treatment and infection control measures to avoid the spread of diseases (Galia *et al.*, 2019). All 78 (100%) bacteriologically positive *S. aureus* isolates were confirmed using *nuc2* gene for *S. aureus* using PCR as shown in (Fig. 1).

Like other bacterial species, *S. aureus* must adhere to host tissue for an infection to develop. Bacterial proteins are the major molecules that interact with host cells and tissues, causing this attachment (Fouad *et al.*, 2022). The initial stage in creating vaccines and diagnostics is the isolation and identification of these proteins. The current work presents two affinity-purified protein bands that were separated from *S. aureus* through affinity column chromatography (CNBr-Sepharose 4B).

The benefit of using CNBr-Sepharose 4B affinity chromatography is that the immunogenic fraction can be purified all at once (Aly *et al.*, 2017). Similarly, *S. aureus* capsular polysaccharide (CP5) was purified using DEAE sephacel ion exchange chromatography (Sigma Chemical Co.). To demonstrate the immunogenicity of the purified element, one-dimensional SDS-PAGE and immunoblot were used after the purification process (Li *et al.*, 2018). In the current study, the confirmed *S. aureus* diagnostic fraction antigen was characterized by two bands at 73.1 KDa and 63.9 KDa described by SDS–PAGE in Figure 2. The two bands of the purified antigen increase the likelihood of detecting antibodies against infection without interference from the eight nonspecific bands of the crude antigen, making the diagnosis using the purified antigen more specific and well-diagnostic than the crude one.

Previously, a 210 KDa molecule was separated by affinity column chromatography and analyzed using SDSPAGE, which revealed extra smaller peptides with fibronectin-binding properties, involving 29 KDa (González-Sapienza *et al.*, 2000). Additionally, among the isolated proteins that have been released by *S. aureus* proteins are 114.8 KDa in size, containing six hydrophobic segments, and 11 KD upon purification with superdex, its immunogenicity was verified through immunoblot (Aly *et al.*, 2017). A proteomic study has identified key proteins in *S. aureus* extracellular vesicles, including penicillin-binding proteins (32 KDa) and bifunctional peptidoglycan hydrolase (138 KDa), and two active proteins: 62 KDa (amidase) and 51 KDa (glucosaminidase) processed from the huge one (Wang *et al.*, 2018).

Based on the result of indirect ELISA reflected by OD readings, the binding activities of fraction antigen were higher than that of crude extract and the binding activities of fraction antigen conjugated gold nanoparticles were higher than that of fraction antigen as shown in Table 3. The separated bands in this investigation demonstrated efficacy in detecting *S. aureus* antigen-specific antibody reactions in milk and serum samples using indirect ELISA, which indicates that these bands have been exhibited during infection and trigger humoral responses in the blood and udder.

The present investigation and its predecessor verified the theory that *S. aureus* exhibits multiple immunogens during infection, whether in blood or milk. On the other hand, the diagnosis of mastitis might be accomplished by employing monoclonal antibodies in ELISA for antigen detection (Fouad *et al.*, 2022). The diagnosis of mastitis involves the detection of antigens or antibodies. It is crucial to note that certain protein molecules are conserved throughout several isolates of *S. aureus*, as demonstrated by Misra *et al.* (2018).

In this study, purified fraction antigen conjugated gold nanoparticles achieved the highest sensitivity to ELISA at 97%, 94%, and 92% at once before storage, 6 months at −20°C, and 1 year at −20°C, respectively. The utilization of purified fraction antigens with gold nanoparticles enhances the sensitivity of ELISA, increasing it from 83.3% to 97%. The obtained result is greater than that of Khodadadi *et al.* (2020), which reported a sensitivity of 93.33% for the developed Nano-ELISA kit. Specific IgG antibodies were detected in 97% (76/78) sera and milk samples related to confirmed *S. aureus* isolates as well as in three cows that revealed positive SCM and negative *S. aureus* isolates bacteriologically and with PCR using gold nanoparticle-based indirect ELISA. This is consistent with Leitner *et al.* (2000), who detected IgG in milk and blood and demonstrated the systemic reaction of all positive isolates following an experimental intramammary *S. aureus* infection. Three negative *S. aureus* that demonstrated little specific IgG antibodies in this study might be related to recently infected and treated cows. The two positive *S. aureus* isolates did not show specific IgG antibodies that might be related to recently infected or immune-suppressed cows.

Researchers have investigated the possibility of utilizing the catalytic properties of AuNPs as a substitute for traditional enzymes used in experiments to improve the sensitivity of ELISA assays. Gold nanoparticles possess extreme stability and exhibit intense peroxidase-like catalytic activity, making them well-suited as nanozymes to enhance the sensitivity of ELISA assays and emerging as alternative options to horseradish peroxidase (HRP) in many biosensing applications (Zhao *et al.*, 2019). Nanoparticles possess a major ratio of surface area to volume, enabling the binding of several enzymes with antibodies (up to 20 HRP per nanoparticle). This results in an enhanced colorimetric signal when compared to a single enzyme-conjugated antibody (Gao *et al.*, 2019).

Zhou *et al.* (2012) found that immobilizing capture antibodies on the AuNPs surface increased their activity by 108% compared to unmodified Elisa plates. This was due to the production of super-hydrophilic surfaces, which improved binding efficiency while preserving antibody activity. The current study establishes a valuable way for *S. aureus* mastitis diagnosis with the use of purified fraction antigen-conjugated gold nanoparticles instead of the traditional way. This will improve the sensitivity and the specificity of ELISA by providing additional binding sites for antibody detection and improving the signal intensity and the stability of biomolecules in complex biological milieus that decrease the reaction time of this assay. The utilization of purified fraction antigen with gold nanoparticles enhances the sensitivity of ELISA from 83.3% to 97% ($p < 0.01$; CI: 99%). The test may be used as a highly sensitive test for monitoring *S. aureus* specific IgG antibodies in milk at 37°C without the need for other laboratory testing. Increasing IgG antibodies for *S. aureus* can be used as a good indicator for early diagnosis of *S. aureus* SCM and subsequently early treatment.

Conclusion

The present study validated the improvement of a novel method for quickly and cost-effectively detecting bovine mastitis using the diagnostic affinity-purified fraction, replacing traditional bacteriological culture and biochemical tests. It is also an important step for infection control. AuNPs serve a variety of purposes, including serving as three-dimensional carriers for antibodies to boost assay sensitivity. They can also replace conventional enzymes to achieve highly sensitive and stable immunological assays. Clinical applications can also utilize AuNPs to create a fast and visually detectable assay. Our study recommends using the optical properties of AuNPs to develop diagnostic systems that allow for visual detection without instruments.

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