Immunoglobulin G-Coated Latex of Rubber Tree (*Hevea brasiliensis*) for Rapid Diagnostic Test for *Staphylococcus aureus*

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ABSTRACT

Antimicrobial resistance (AMR) poses a significant global health threat, with *Staphylococcus aureus*—particularly methicillin-resistant strains (MRSA)—being a major contributor to high mortality rates. Rapid and accurate diagnostic methods are essential for early detection and effective treatment. This study aimed to evaluate immunoglobulin G (IgG)- coated latex derived from the rubber tree (*Hevea brasiliensis*) as a rapid diagnostic tool for detecting *S. aureus* in an experimental design. Latex was extracted and processed to obtain a cream fraction with a 35.19% composition, which was found sufficient for effective application. Various concentrations of IgG-coated latex (25%, 50%, 75%, and 100%) were tested against *S. aureus*. Among them, the 75% concentration produced the most distinct agglutination, balancing both reaction speed and clarity. Results also showed that all concentrations induced agglutination, with reaction times ranging from 1:15 to 2:19 minutes. While the 100% concentration produced the fastest agglutination time, the reaction was less visually pronounced due to its higher density. The test's specificity was confirmed using negative controls—*Pseudomonas aeruginosa, Escherichia coli*, and distilled water—which showed no agglutination. These findings demonstrate that IgG-coated latex, particularly at 75% concentration, produces a rapid, specific, and visually clear agglutination reaction with *S. aureus*. This highlights its potential as a reliable tool for the rapid and accurate detection of *S. aureus* in clinical diagnostics.

Keywords: agglutination, cream fraction, dilution, inoculation, ultracentrifugation

INTRODUCTION

Background of the Study

Antimicrobial resistance (AMR) is a global public health threat, causing over 5 million deaths and directly contributing to 1.27 million in 2019. *Staphylococcus aureus* (*S. aureus*), a gram-positive bacterium commonly found in the human body, is a major contributor to antibiotic resistance-related deaths. It can cause a range of illnesses, from skin infections to serious conditions like bacteremia (An et al., 2024).

Methicillin-resistant *S. aureus* (MRSA) is a leading cause of healthcare-associated infections and was the top pathogen-drug combination in AMR cases in 2019. Effective AMR surveillance—from local to global levels—is essential for identifying resistance trends, guiding prevention strategies, and improving treatment guidelines (An et al., 2024).

Rapid diagnostic tests (RDTs) with turnaround times of under 2 hours have been widely introduced in labs and emergency settings to enable quick detection and treatment. Their success in antimicrobial stewardship and patient care depends on their performance and clinical impact (Bouzid et al., 2020). Although proven effective, current rapid diagnostic tests face a critical challenge: providing cost-effective, easy-to-use technology for healthcare and diagnostics (Hassan et al., 2020). In a study by Eldin et al. (2019), the presence of several unwanted reactions caused by genes common to various bacterial species poses a significant obstacle to bacterial serology.

The latex agglutination test, favored for its speed, works by detecting antigen-antibody reactions using latex beads—small polystyrene particles coated with antibodies or antigens— to identify biological targets in samples (Mahat et al., 2014). Thus, there is a need to develop rapid, specific, natural, and cost-effective diagnostic methods to efficiently manage S. aureus infections. IgG-coated latex particles could bridge the gap by providing rapid results via a simple agglutination procedure, ensuring high specificity, particularly for S. aureus detection, and enabling the maximum use of IgG antibodies, which can eliminate the need for additional reagents. Furthermore, rapid detection of MRSA in cases of bacteremia and immediate reporting of results can improve the diagnosis of bloodstream infections and patient treatment.

A rapid test for *S. aureus* would greatly improve health outcomes by facilitating faster identification, preventing antibiotic resistance, limiting the spread of infections, and avoiding the needless use of antibiotics. Better treatment and infection control, particularly in places with limited resources, depend on rapid, simple tests, as current diagnostic techniques are often slow and require specialist equipment. This alternative screening test improves early identification and treatment, which aligns with the UN Sustainable Development Goal 3: Good Health and Well-Being.

Statement of the Objectives

This study aimed to evaluate the immunoglobulin G-coated latex of the rubber tree (Hevea brasiliensis) as a rapid diagnostic test for *S. aureus*. This study was conducted in January- March 2025.

Specifically, it sought to answer the following problems:

1. To determine the percent composition of the cream fraction in a given mass of the latex sample.
2. To determine the optimum concentration that gives the optimum binding for IgG-coated latex.
3. To determine the agglutination time of IgG-coated latex against *S. aureus.*

METHODOLOGY

Research Design

The study employed an experimental design that incorporated several fundamental laboratory procedures and setups. Key methodologies include ultracentrifugation, bacterial cultivation, latex binding with IgG, and slide testing using reverse passive agglutination. Such procedures were utilized to determine the percent composition of cream fraction in a given mass of the latex sample, the optimum concentration that gives the optimum binding for IgG-coated latex, and determine the agglutination time of IgG-coated latex against *S. aureus.*

Study Site and Sample Collection

The study was conducted at Saint Mary's University in Bayombong, Nueva Vizcaya. *S. aureus* was obtained and stored at Saint Mary's University's Center for Natural Sciences Research Laboratory. Latex from the rubber tree (*Hevea brasiliensis*) was gathered at Diadi, Nueva Vizcaya, while the Human Normal IgG (Immunorel) was sourced from Region 2 Trauma and Medical Center (R2TMC) at Bayombong, Nueva Vizcaya, Philippines. All laboratory procedures were performed at Saint Mary's University's Center for Natural Sciences Research Laboratory, except for latex cream fraction ultracentrifugation performed at the Region 2 Trauma and Medical Center (R2TMC) Laboratory.

Specimen Certification

Before the collection of the latex from the rubber tree (*Hevea brasiliensis)*, a leaf or bark from the tree was submitted to the Nueva Vizcaya State University College of Forestry, Environment and Resources Management for the authentication and certification of its botanical or taxonomic identity. While the *S. aureus* and the negative controls, namely *Pseudomonas aeruginosa* and *Escherichia coli,* were obtained from the Center for Natural Sciences of Saint Mary's University, and their identity was verified by the laboratory assistant

Data Gathering Procedure

Collection of Latex Sample and Centrifugation

Latex from *Hevea brasiliensis* (clone RRIM 600) trees in Diadi, Nueva Vizcaya, was collected early in the morning (5:00–6:00 a.m.) by cutting a 6.4 mm deep groove into the bark to allow latex to flow into a collecting cup (Freudenrich, 2023). The harvested latex was preserved with 0.01–0.1% ammonia solution, filtered through cheesecloth, and stored in an ice box to maintain stability (Jawjit et al., 2015). After collecting the latex samples, three conical tubes were each filled with 9 mL of latex and subjected to ultracentrifugation. The samples were centrifuged at 16,000 g for 45 minutes at 4 °C, followed by 41,000 g for an additional 45 minutes, yielding three distinct layers. According to Bottier (2019), the top layer contains large rubber particles (cream), the middle layer has small rubber particles (skim) and Frey-Wyssling particles in C-serum, and the bottom layer consists of lutoids. The procedure was conducted at the Region 2 Trauma and Medical Center (R2TMC) Blood Bank laboratory.

Determination of Percent Composition of the Cream Layer Portion

After the separation period, the cream layer, which consisted predominantly of rubber particles, was carefully siphoned or decanted using a pipette or an appropriate tool to avoid disturbing the serum layer. The cream fraction from each conical tube was isolated and weighed individually. The volume or weight of the cream layer was recorded. Where conical tube A weighed 2.9 grams, conical tube B weighed 3.8 grams, and conical tube C weighed 2.8 grams, which garnered a total volume of 9.5 grams of cream fraction. The percent composition of the cream layer was calculated using the formula from LibreTexts (2021):

Cultivation of S. aureus in MSA

Mannitol Salt Agar (MSA) is a selective medium used to isolate and quantify coagulase-positive *Staphylococcus aureus*. It contains beef extract, proteose peptone, mannitol, sodium chloride, phenol red, and agar, with a final pH of 7.4 ± 0.2. The high salt concentration (7.5% NaCl) inhibits most bacteria except staphylococci, while mannitol serves as a fermentable carbohydrate. To prepare MSA, 111 g of powder was dissolved in 1 L of water, boiled, and sterilized at 121°C for 15 minutes. *S. aureus* was streaked on the medium and incubated at 35 ± 2°C for 24–48 hours, producing yellow colonies due to mannitol fermentation (Aryal, 2022).

Coating of Latex (cream layer) with Immunoglobulin G

Human Normal Immunoglobulin (ImmunoRel) is a sterile, solvent/detergent-treated IgG solution derived from pooled human plasma. It is produced using an adapted Cohn fractionation process followed by ultrafiltration and ion-exchange chromatography, ensuring viral safety and preserving antibody activity. The IgG retains its Fc portion and natural antibody functions against bacteria and viruses, with a subclass distribution similar to that of normal plasma and IgA ≤80 mg/L. Maltose (100 g/L) acts as a stabilizer (Reliance Life Sciences, 2022).

For latex sensitization, 200 μL of latex beads were mixed with 200 μL of glycine saline buffer and anti-human IgG, incubated at 37°C for 1.5 hours, then centrifuged at 5000 rpm for 10 minutes. After washing and incubation in blocking buffer for 72 hours at 4°C, the buffer—typically containing BSA, casein, or non-fat milk—prevented non-specific protein binding, ensuring specific antibody-antigen interactions (Ravi et al., 2009).

Preparation of Concentration

To determine the optimal binding concentration, the IgG-coated latex was diluted to 25%, 50%, 75%, and 100% in Normal Saline Solution (NSS). A stock solution of IgG-coated latex at 100% concentration served as the base for all preparations. The 100% concentration was used undiluted, while the 75% concentration was prepared by mixing three parts of the stock solution with one part of NSS. For the 50% concentration, equal volumes of the stock solution and NSS were combined; for the 25% concentration, one part of the stock solution was mixed with three parts of NSS. Each dilution was prepared in clean, labeled test tubes and gently mixed to ensure uniformity (Cullen & MacIntyre, 2016).

Slide test: Direct Inoculation of the S. aureus to the IgG-coated Latex Particles

Staphylococcus aureus was tested alongside three negative controls: *Pseudomonas aeruginosa*, *Escherichia coli*, and distilled water. *P. aeruginosa* is an antibiotic-resistant opportunistic pathogen often found with *S. aureus* in chronic infections and classified by the WHO as a critical ESKAPE pathogen requiring new treatments (Briaud et al., 2022; Qin et al., 2022). *E. coli* and *S. aureus* are major causes of hospital-acquired infections and bacteremia, responsible for over half of all community and hospital cases (Poolman & Anderson, 2018). Distilled water served as an inert negative control to prevent experimental bias (Brenner, 2022).

Equal bacterial colonies were inoculated into 2 mL of normal saline solution (0.85–0.90%), a recommended diluent that maintains microbial cell integrity (CLSI). For the slide agglutination test, 50 μL of varying IgG-coated latex concentrations was mixed with 50 μL of each bacterial suspension on separate slides. The reaction was observed in three trials for consistency. *S. aureus* showed visible clumping, indicating a positive antigen–antibody reaction, while *P. aeruginosa*, *E. coli*, and distilled water showed no reaction, confirming the test's specificity (LibreText).

Rapid Diagnostic Test for S. aureus

The reaction was monitored for clumping, which signified a positive result indicating the presence of *S. aureus*. The slides were gently tilted to visualize the clumps, and the agglutination time was recorded. Agglutination occurs when latex particles coated with antibodies bind to antigens in the sample, forming visible clumps. This process followed the principle of reverse passive agglutination, in which antibodies—rather than antigens—are attached to carrier particles, such as latex beads. When the target antigen is present, it binds to these antibodies, causing agglutination visible to the naked eye. According to Stevens (2016), this method enables rapid and accurate antigen detection and is widely used in clinical diagnostics due to its high sensitivity and specificity.

Treatment of Data

The results of the clumping were analyzed qualitatively utilizing an agglutination grading system

Table 1

Agglutination Grading System

4+	One solid aggregate of cells
3+	Two to three large aggregates of cells
2+	Several medium-sized clumps
1+	Several small clumps
W+	(Weak reaction) = many tiny agglutinates
Negative	No aggregation

The agglutination grading system was based on the laboratory manual in Serology and Immunology: Basic Concepts, Principles, and Procedures by Domingo et al.

Ethical Considerations

The study underwent ethical review by Saint Mary's University Research Ethics Board Institutional Biosafety Committee (SMUREB-IRC-2025 0865), including the address and contact details on the 2nd floor, Rev. John Van Bauwel Hall, Saint Mary's University Main Campus, Ponce Street, Don Mariano Marcos, Bayombong, 3700 Nueva Vizcaya, Philippines (email: reb@smu.edu.ph; cellphone: 09177053041).

RESULTS AND DISCUSSIONS

Section 1. Percent Composition of Cream Fraction

The cream fraction extracted from 27 milliliters of pure latex yielded 9.5 grams, which corresponds to approximately 35.19% of the total latex composition. This considerable yield demonstrates the effectiveness of the extraction method employed. The recovered cream fraction was subsequently utilized in testing *Staphylococcus aureus* across three separate trials. Each trial included the use of negative controls to ensure the accuracy and reliability of the experimental outcomes.

Therefore, the quantity of cream fraction obtained in this study was not only within the expected yield range but also sufficient to support multiple rounds of experimental assays. The amount extracted proved adequate for repeated testing and provided reliable material for antimicrobial evaluation. This indicates potential for scaling the method for future research or applications involving latex-derived bioactive components

Section 2. Optimum Concentration That Gives the Optimum Binding for IgG Coated Latex

Table 2

Slide Test Result for Agglutination Reaction of Staphylococcus aureus

	Staphylococcus aureus		
Concentration	**Trial 1**	**Trial 2**	**Trial 3**
25%	W+	W+	W+
50%	1+	W+	W+
75%	**1+**	**1+**	**1+**
100%	W+	W+	1+

S. aureus exhibited positive agglutination across all tested concentrations of IgG- coated latex from the Rubber Tree, with the 75% concentration showing the most visibly pronounced agglutination due to its increased opacity. This result was based on the manner of reporting written in the Laboratory Manual in Serology and Immunology by Domingo et al. (2022).

This finding correlates with studies showing that IgG binds to S. aureus protein A, a mechanism highlighted by Choe et al. (2016), who discuss how Protein A binds specifically to the Fc region of immunoglobulins. For *S. aureus*, it was expected to have the said reaction. Protein A (SpA), a pathogenic component produced by *S. aureus*, is widely used in antibody purification techniques, such as chromatography, due to its strong and specific binding to IgG antibodies from a variety of animal species. It is well established that SpA interacts solely with the Fc portion of antibodies and is used in double-sandwich immunoassays, immunoprecipitation, and antibody affinity purification.

Therefore, the IgG-coated latex is effective at inducing agglutination in S. aureus, with higher concentrations improving both the visibility and the speed of the reaction, making it a suitable choice for rapid, accurate detection.

Table 3

Slide Test Result for Agglutination Reaction of Negative Controls

Concentration	Negative Controls								
	Pseudomonas aeruginosa			***Escherichia coli***			**Distilled Water**		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
25%	0	0	0	0	0	0	0	0	0
50%	0	0	0	0	0	0	0	0	0
75%	0	0	0	0	0	0	0	0	0
100%	0	0	0	0	0	0	0	0	0

Legend: T1 = Trial 1; T2 = Trial 2; T3 = Trial 3

As observed, all values recorded in the table are zero, indicating that there was no reactivity or positive response in any of the negative controls at any concentration level. This consistency across all replicates and bacterial species confirms the specificity of the diagnostic test. In other words, the Immunoglobulin G (IgG)-coated latex did not exhibit cross-reactivity with non-target bacteria such as *P. aeruginosa* and *E. coli*, nor did it produce false positives when exposed to distilled water. This is a crucial validation step to ensure that the rapid test reacts exclusively to the target pathogen, *S. aureus*.

The results for *P. aeruginosa* and *E. coli* as negative controls showed no agglutination across all concentrations and trials, reinforcing the test's specificity. According to Biology Insights Team (2025*), P. aeruginosa* produces Exotoxin A as a virulence factor, which does not interact with IgG, explaining the absence of a reaction. Similarly, Ghumra and Pleass (2007) reported that *E. coli* lacks Fc-receptors for human IgG, further supporting the lack of agglutination. These findings confirm that the IgG- coated latex test does not produce nonspecific reactions with non-target bacteria.

The complete absence of positive results in all negative control trials strongly supports the specificity and reliability of the developed rapid diagnostic test. These findings suggest that the IgG-coated latex does not bind nonspecifically to other common bacterial species or inert liquids, thereby minimizing the risk of false positives in practical applications. This strengthens the overall credibility of the study and indicates a promising potential for real-world clinical or field deployment, particularly in settings where quick and accurate detection of *S. aureus* is essential for infection control and treatment.

Therefore, the results for *P. aeruginosa*, served as a negative control, show no agglutination at any concentration or trial, as expected. Biology Insights Team (2025), states that *P. aeruginosa* only has a virulent factor, Exotoxin A, which is not the primary characteristic that specifically reacts with IgG. The lack of agglutination supports the test's specificity, confirming that no nonspecific interactions occurred.

Table 4
Slide Test Result for Agglutination Time of Staphylococcus aureus

	Staphylococcus aureus			
Concentration	**Trial 1**	**Trial 2**	**Trial 3**	**Average**
25%	2:17	2:10	2:19	2:15
50%	2:10	2:08	2:08	2:09
75%	**1:54**	**2:09**	**2:07**	**2:03**
100%	1:51	2:05	1:56	1:57

The table displays agglutination times (in minutes) for *S. aureus* using various concentrations of IgG-coated latex (25%, 50%, 75%, and 100%). The results are recorded across three trials, and an average is calculated for each concentration. As the concentration of IgG-coated latex increases, the agglutination time decreases. This suggests that higher concentrations of IgG-coated latex enhance the efficiency of the antigen-antibody reaction, resulting in faster agglutination.

The results correlate with established immunological principles, in which higher antibody concentrations, such as IgG, increase the likelihood of binding to specific antigens, such as those on *S. aureus*. This elevated interaction frequency accelerates the cross-linking of particles, resulting in faster and more efficient agglutination as highlighted by Choe et al. (2016). The scientific literature supports the notion that optimal antibody density is crucial for balancing sensitivity and visual clarity in agglutination assays. This validates the experimental finding that 75% IgG-coated latex is ideal for effective, observable rapid diagnostics, and this result was based on the reporting format outlined in the Laboratory Manual in Serology and Immunology by Domingo et al. (2022).

With these outcomes, it was found that all concentrations exhibited rapid agglutination times ranging from 1:51 to 2:19. The 100% concentration not only induced agglutination the fastest but, due to its excessive density, the agglutination was not as visibly pronounced. In contrast, the 75% concentration showed the most clearly visible agglutination, making it highly suitable for rapid diagnostic testing of *S. aureus*. Therefore, the data demonstrate that increasing the concentration of IgG-coated latex significantly reduces the agglutination time in detecting *S. aureus*.

Figure 3

Agglutination Reaction of Human IgG-coated latex of Rubber Tree with Staphylococcus aureus

Small clumps at the periphery of the solution, graded as 1+, were observed in all three trials. Although these observations were observed only with 50%, 75%, and 100% IgG-coated latex, 75% showed the most promising agglutination, consistent with a 1+ grade. Moreover, many tiny agglutinates, graded as W+, were commonly observed in 25% of concentration. With this outcome, it was found that either of the different IgG-coated latex concentrations produced an agglutination reaction, and, according to Domingo et al. (2022), this indicated the presence of antigen-antibody reactions. In this study, S. aureus and IgG-coated latex bind together, causing agglutination during the slide test procedure.

The result of the slide test is supported by the principle of latex of agglutination test discussed by McPherson and Pincus (2021), this test relies on antibody antigen interactions whether it is the antibodies or antigen that will create a reaction with the latex reagent the visible agglutination are clumping in the latex slight test suggest that there was a presence of antigen antibody reaction therefore the antibodies embedded within the latex was able to bind with the antigen as observed during the experiment since there was an agglutination reaction.

All laboratory procedures, including waste disposal, were conducted with the guidance of laboratory assistants, while the results were validated by a registered medical technologist.

CONCLUSIONS AND RECOMMENDATIONS

Conclusion

This study sought to utilize the biocompatibility and binding properties of natural latex as a sustainable and effective alternative to synthetic carriers in immunoassays, specifically to develop a rapid diagnostic test for *Staphylococcus aureus*. The study confirmed that the cream fraction obtained—9.5 grams, accounting for 35.19% of the total latex volume—was sufficient for effective application. Latex particles were coated with various concentrations of IgG antibodies and tested for agglutination with *S. aureus*. Results showed that the 75% IgG-coated latex consistently produced a 1+ agglutination response across all trials, demonstrating strong binding performance and reliability in detecting *S. aureus*. While all tested concentrations showed positive agglutination, the 75% concentration generated the most pronounced reaction, with an average completion time of 2 minutes and 3 seconds. These findings suggest that IgG-coated natural latex is both effective and efficient at inducing agglutination of S. aureus, making it a promising option for rapid, accurate detection.

Recommendations

1. Future researchers may improve the diagnostic accuracy for *S. aureus* by employing specific IgG antibodies and investigating the use of the latex cream fraction in rapid diagnosis for other *Staphylococcus* species, thereby reducing cross-reactivity and false positive reactions.

2. To further evaluate optimization of IgG coating with various coating techniques and buffer conditions to optimize IgG binding efficiency on latex particles, ensuring consistent and stable antibody attachment and improving its stability.

3. It is recommended to identify the factors that lead to false positives and negatives in order to establish the optimal conditions for achieving the most rapid and distinct agglutination response.

4. Further evaluation of the reagent through direct inoculation using clinical samples, including skin wound exudates, pus, and serum, is recommended to validate its efficacy

and diagnostic reliability.

5. It is also recommended to include a confirmatory test in rapid diagnostic assays for *S. aureus* to verify results and reduce false positives and negatives, improving the overall sensitivity and specificity of the diagnostic test.

REFERENCES

An, N., Hai, L., Luong, V., Vinh, N., Hoa, P., Hung, L., Son, N., Hong, L. T., Hung, D., Kien, H., Le, M., Viet, N., Nguyen, D., Pham, N., Thang, T., Tien, T., & Hoang, L. (2024). *Antimicrobial resistance patterns of Staphylococcus aureus isolated at a general hospital in Vietnam between 2014 and 2021.* Infection and Drug Resistance, 17, 259–273. https://doi.org/10.2147/idr.s437920

Aryal, S. (2022, August 10). *Mannitol Salt Agar for the isolation of Staphylococcus aureus.* MicrobiologyInfo.com. https://microbiologyinfo.com/mannitol-salt-agar-for-the-isolation-of-staphylococcus-aureus/

Biology Insights Team. (2025, January 16). *Pseudomonas Exotoxin A: Structure, Action, and Pathogenic Role.* Biology Insights. https://biologyinsights.com/pseudomonas-exotoxin-a-structure-action-and-pathogenic-role/

Bottier, C. (2019). *Biochemical composition of Hevea brasiliensis latex: A focus on the protein, lipid, carbohydrate and mineral contents.* In *Advances in Botanical Research,* (pp. 201–237). https://doi.org/10.1016/bs.abr.2019.11.003

Bouzid, D., Zanella, M., Kerneis, S., Visseaux, B., May, L., Schrenzel, J., & Cattoir, V. (2020). *Rapid diagnostic tests for infectious diseases in the emergency department.* Clinical Microbiology and Infection, *27*(2), 182–191. https://doi.org/10.1016/j.cmi.2020.02.024

Brenner, L. (2022, August 30). *Why is distilled water a good control for science projects?* Sciencing. https://www.sciencing.com/distilled-good-control-science-projects-7418493/

Briaud, P., Camus, L., Bastien, S., Doléans-Jordheim, A., Vandenesch, F., & Moreau, K. (2019). *Coexistence with Pseudomonas aeruginosa alters Staphylococcus aureus transcriptome, antibiotic resistance and internalization into epithelial cells.* Scientific Reports, *9*(1). https://doi.org/10.1038/s41598-019-52975-z

Choe, W., Durgannavar, T., & Chung, S. (2016). *Fc-binding ligands of immunoglobulin G: An overview of high affinity proteins and peptides.* Materials, *9*(12), 994. https://doi.org/10.3390/ma9120994

Cullen, J. J., & MacIntyre, H. L. (2016). *On the use of the serial dilution culture method to enumerate viable phytoplankton in natural communities of plankton subjected to ballast water treatment.* Journal of Applied Phycology, *28*(1), 279–298. https://doi.org/10.1007/s10811-015-0601-x

Domingo, J. D., Cardona, C. C., Flores, A. R. F., Martín, G. L., & García-Meim, R. S. P. (2022). *Laboratory manual in serology and immunology: Basic concepts, principles, and procedures* (pp. 25–30). C&E Publishing, Inc.

Eldin, C., Parola, P., & Raoult, D. (2019). Limitations of diagnostic tests for bacterial infections. *Médecine et Maladies Infectieuses*, *49*(2), 98–101. https://doi.org/10.1016/j.medmal.2018.12.004

Freudenrich, C., PhD. (2023, August 18). *How rubber works.* HowStuffWorks. https://science.howstuffworks.com/rubber1.htm

Ghumra, A., & Pleass, R. J. (2007). Escherichia coli do not express Fc-receptors for human immunoglobulin G (IgG). *Molecular Immunology, 44*(8), 2144–2146. https://doi.org/10.1016/j.molimm.2006.09.019

Hassan, S., Donia, A., Sial, U., Zhang, X., & Bokhari, H. (2020). Glycoprotein- and lectin-based approaches for detection of pathogens*. Pathogens, 9*(9), 694. https://doi.org/10.3390/pathogens9090694

Jawjit, W., Pavasant, P., & Kroeze, C. (2013). *Evaluating the environmental performance of concentrated latex production in Thailand.* Journal of Cleaner Production, 98, 10–16. https://doi.org/10.1016/j.jclepro.2013.11.016

McPherson, R. A., & Pincus, M. R. (2021). *Henry's Clinical Diagnosis and Management by Laboratory Methods* (24th ed.). Elsevier.

Poolman, J. T., & Anderson, A. S. (2018). Escherichia coli and Staphylococcus aureus: Leading causes of hospital-acquired infections. *Clinical Microbiology Reviews, 31*(3), e00083–17. https://doi.org/10.1128/CMR.00083-17

Qin, N., Tan, X., Jiao, Y., Liu, L., Zhao, W., Yang, S., & Chen, Y. (2022). *Interactions between Staphylococcus aureus and Pseudomonas aeruginosa in polymicrobial infections.* Frontiers in Microbiology, 13, 886374. https://doi.org/10.3389/fmicb.2022.886374

Ravi, S., Gunasekaran, B., & Subramanian, S. (2009). Latex agglutination test for the detection of specific antigens and antibodies. *Indian Journal of Medical Microbiology, 27*(1), 46–49. https://doi.org/10.4103/0255-0857.45360

Reliance Life Sciences. (2022). *ImmunoRel: Human normal immunoglobulin.* https://www.rellife.com/products/immunorel

Stevens, C. D. (2016). *Clinical immunology and serology: A laboratory perspective* (4th ed.). F.A. Davis Company.