ORIGINAL ARTICLE

Smart Medical Journal

Smart Medical Journal *(SMedJour)* August 2023, Vol. 6, No.2, pp: 67-71
DOI : https://doi.org/10.13057/smj.v6i2.68910
E-ISSN: 2621-0916 | P-ISSN: 2621-1408

Modification of Secondary Dyes in Gram Staining Protocol to Increase The Diagnostic Accuracy of *Bacterial Vaginosis*

Rahma Maulida Risqia[1], Maryani[2*], Alamanda Murasmita[3]

*Corresponding author: maryani66@staff.uns.ac.id

Affiliation:
[1]Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia
[2] Department of Microbiology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia
[3] Department of Dermatology and Venereology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia

Recived: 20/12/2022
Accepted: 08/05/2023
Published: 31/08/2023

ABSTRACT

Introduction: The diagnosis of bacterial infection can only be made by gram stain with patient information. One of the problems with gram staining is that the resulting microscopic images look dim, making them prone to misinterpretation. Researchers conducted a study on modifying secondary dyes in gram staining to improve the accuracy of the diagnosis of *Bacterial vaginosis* infections.
Methods: This research is a laboratory experimental study with a cross sectional approach. The sample of this study consisted of anaerobic gram-negative rods on vaginal swabs of patients diagnosed with *Bacterial vaginosis* at Dr. Moewardi Hospital and Sebelas Maret University Hospital during March to July 2022. The samples were then treated in the form of gram staining with different secondary dyes and then compared.
Results: The color intensity of anaerobic gram-negative rod bacteria samples treated with gram staining using fuchsin secondary dye appeared to be stronger than safranin secondary dye. Likewise, the resulting focus looks clearer. The diagnostic test showed that modification of secondary dyes in gram staining by substituting safranin into fuchsin increased the color intensity and focus of preparations
Conclusion: Modification of secondary dye on gram staining by replacing secondary dye of safranin to fuchsin can improve the quality of gram staining results on anaerobic gram negative bacteria thereby increasing the accuracy of diagnosis and treatment given.

Keywords: secondary gram staining; diagnosis of infection; anaerobic gram negative rods bacteria; *Bacterial vaginosis*

INTRODUCTION

Infectious disease is a health problem in the world, including in Indonesia[1]. One example of an infectious disease caused by anaerobic bacteria is *Bacterial vaginosis*. Infection occurs because there is an imbalance in the amount of normal vaginal flora with anaerobic bacteria that are found[2]. Based on this, bacterial staining is a reliable method for determining the etiological diagnosis of infectious diseases[3].

Microbiological diagnosis in cases of bacterial infection can be enforced simply by gram staining accompanied by information from the patient. The accuracy obtained can reach 70-90%[4]. One of the problems with gram staining is that the resulting microscopic image looks faint or thin, making it prone to misinterpretation, especially for gram-negative bacteria. The gram stain error rate varies from 0.4% to 2.7%[5]. The error in interpreting the gram stain results alone can reach 6.4% with an average error rate of 2.9%[6].

The secondary dye commonly used in gram staining is safranin. In gram staining, gram-negative bacteria will change color to pink after being stained with safranin. The use of safranin in gram stain produces a color that lacks contrast[7]. Besides safranin, fuchsin is a dye that can give gram-negative bacteria a pink color. The pink color produced by fuchsin appears more apparent when compared to safranin[8].

Gram staining using standard secondary dyes appears faint when observed using a microscope, so the diagnosis and management of infectious diseases become less effective. The researcher modified the secondary dye in gram staining from a standard secondary dye in the form of safranin to fuchsin. This is expected to improve the quality of gram stain results so that the diagnosis of infectious diseases and antibiotic therapy can be more precise and efficient. Based on the explanation above, the researcher conducted a study on modifying secondary dyes in gram staining to improve the accuracy of the diagnosis of *Bacterial vaginosis* infections.

METHODS

This type of research is a laboratory experiment with a cross-sectional approach. Sampling was done using a total sampling technique from March to July 2022 at Dr. Moewardi Hospital and Sebelas Maret University Hospital. The research location is in the Microbiology Laboratory, Faculty of Medicine, Sebelas Maret University. The sample of this study was anaerobic gram-negative rods on vaginal swabs of patients diagnosed with *Bacterial vaginosis*.

Then each sample was made into two preparations. Preparations from the same sample were then separated and given different treatments. In the first sample preparation, gram staining was carried out with crystal violet primary dye, iodine mordant, distilled water bleach, and safranin secondary dye. In contrast, gram staining was performed with crystal violet primary stain, iodine mordant, distilled water bleach, and fuchsin secondary stain in the second sample preparation. After that, the gram-staining results of the control and treatment groups were compared, including the color intensity and focus of anaerobic gram-negative rods.

Data analysis was done by diagnostic test. This study received ethical approval from the Health Research Ethics Committee Dr. Moewardi Hospital, on March 21 2022, with number 359/III/HREC/2022 accompanied by informed consent for patients whose vaginal swab samples were used in this study.

RESULTS

In this study, a sampling of anaerobic gram-negative rod bacteria used the total sampling method from March to July 2022 and obtained 10 samples gram-negative bacterial infections from vaginal swab patients diagnosed with *Bacterial vaginosis*.

Table 1. Distribution of anaerobic gram-negative rod samples

Sample	Frequency	Percentage(%)
Vaginal swab	10	100
Total	10	100%

Table 2. Diagnostic test for secondary dye modification in gram staining

Gram Staining		Secondary Dyes Safranin		
		BV (+)	BV (-)	Total
Secondary Dyes Fuchsin	BV (+)	5	1	6
	BV (-)	1	3	4
	Total	6	4	10

Based on the research that has been done, obtained data regarding the results of gram staining in the sample. Data were obtained by comparing the results of gram staining in each sample whose status was grouped based on the secondary dyes used in gram staining, namely safranin and fuchsin**.**

Table 3. Result of diagnostic test for secondary dye modification in gram staining

Diagnostic Test	Diagnostic Result
Sensitivity	83,3%
Specificity	75%
Positive Likehoold Ratio	3,33
Negative Likehoold Ratio	0,66
Pretest Probabilty	60%
Pretest Odds	1,5
Posttest Odds	4,99
Posttest Probabilty	83,3%

Based on the diagnostic results in the table above, a sensitivity value of 83,3% and a specificity of 75% were obtained. In addition, the difference between 60% pretest probability and 83,3% posttest probability indicates that modifying the secondary dye in gram staining by substituting the secondary dye from safranin to fuchsin can improve the quality of gram staining preparations. This is useful in increasing the accuracy of diagnosing and managing infectious diseases caused by gram-negative anaerobic rods.

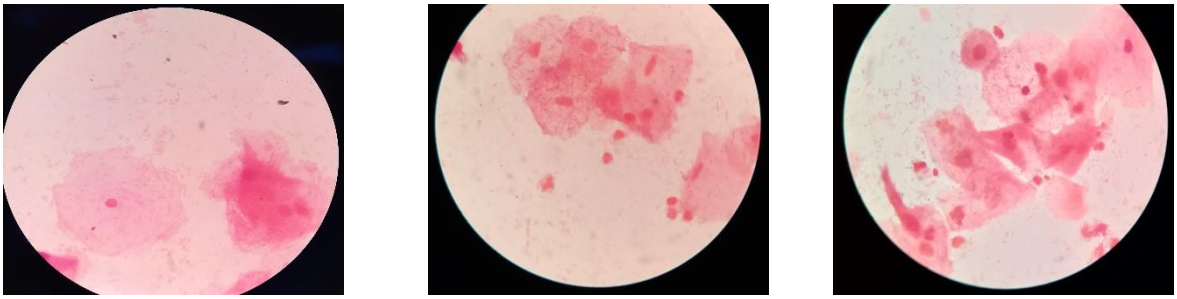

Figure 1. Results of gram staining of *Bacterial vaginosis* samples using safranin

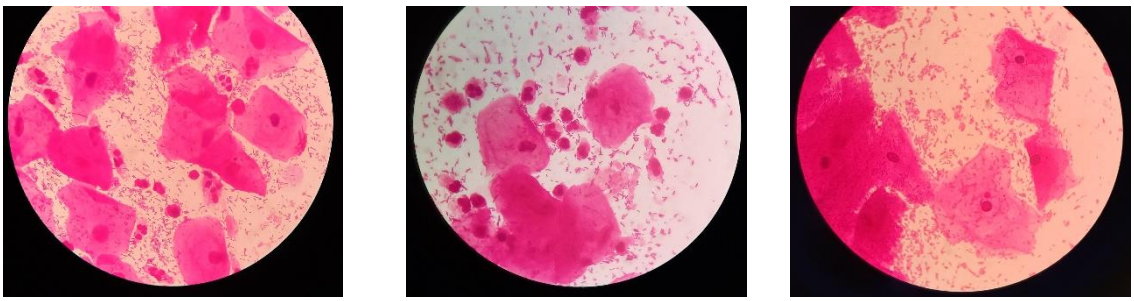

Figure 2. Results of gram staining of *Bacterial vaginosis* samples using fuchsin

DISCUSSION

Anaerobic bacteria are part of the normal flora in the human body, especially on the skin and mucous membranes. Infections caused by anaerobic bacteria can occur due to disruption of the

mucocutaneous barrier or immunosuppression. Anaerobic bacteria can be found in vaginal discharge patients diagnosed with *Bacterial vaginosis*. Diagnosis of infections caused by anaerobic bacteria requires the assistance of an appropriate microbiologist[3].

Gram staining can be used to identify infections caused by anaerobic bacteria so that the diagnosis of infectious diseases can be established appropriately. Interpretation of gram staining preparations plays a vital role in controlling anaerobic bacterial infectious diseases[9]. There is a significant 9% to 45% misinterpretation of gram stain preparations. Incorrect interpretation of Gram stain can harm patient care[5]. However, if done correctly, gram stain interpretation will assist in diagnosing infectious diseases so that initial therapy can be given more quickly, precisely, and efficiently[9].

In this study, secondary dyes were modified in gram staining by comparing the results of gram staining of anaerobic gram-negative rods using secondary dyes of safranin and fuchsin. Based on the results of the study, it was found that the resulting color intensity and focus looked better in the staining results using fuchsin secondary dyes. Gram staining of anaerobic gram-negative rods using fuchsin secondary dyes produces a more intense image when observed under a microscope. This can happen because the affinity of the fuchsin color for the cell wall of gram-negative bacteria is better so that the absorption of the color that occurs is also more perfect[8].

Microscopic examination of vaginal swab treated with gram staining is beneficial in diagnosing infectious diseases, especially those caused by gram-negative anaerobic rods. Identifying infectious diseases by gram staining of vaginal swab can determine the use of appropriate antibiotic therapy so that antibiotic resistance can be prevented. Gram staining techniques can be used to assist in the diagnosis of *Bacterial vaginosis* with Nugent scoring[12]. Nugent scoring is a standard quantitative morphological classification method for establishing the diagnosis of *Bacterial vaginosis*. The vaginal swab smear treated with gram staining was then observed under a microscope and counted the number of gram-negative anaerobic rods found was. Scores of 0-3 represent normal vaginal flora, scores of 4-6 represent moderate vaginal flora, and scores of 7-10 are considered diagnostic for *Bacterial vaginosis*[13].

Apart from being determined by counting the number of gram-negative rods, another clinical criterion for establishing the diagnosis of *Bacterial vaginosis* is finding clue cells in vaginal swab preparations. Clue cells are vaginal epithelial cells surrounded by gram-negative rods[14]. Based on research that has been done, gram-negative rods and clue cells found in gram-staining vaginal swab preparations using secondary dye fuchsin have sharper color intensity and better focus. This is very helpful in establishing a quantitative diagnosis of *Bacterial vaginosis* by counting the number of gram-negative bacteria found on microscopic examination or by observing the clue cells found[13]. The weakness of this study is that it only uses secondary dyes with a concentration of 0.5%, so the quality of the modified gram stain using secondary dyes with other concentrations is unknown.

CONCLUSION

Modification of the secondary dye in gram staining by substituting the secondary dye from safranin to fuchsin can improve the quality of gram stain results on anaerobic gram-negative rods, thereby increasing the accuracy of the diagnosis of anaerobic gram-negative rod infections and their management.

ACKNOWLEDGMENTS

The author would like to thank to Marwoto, dr., Sp.MK., M.Sc. who gave suggestions in conducting this research.

CONFLICT OF INTEREST

The authors reported no competing interests.

REFERENCE

1. Aniq A, Mutsaqof N, Suryani E, Kom SSM. Sistem Pakar Untuk Mendiagnosis Penyakit Infeksi Menggunakan Forward Chaining. ITSMART. 2015;4(1):1–5.
2. Africa CWJ, Nel J, Stemmet M. Anaerobes and Bacterial Vaginosis in Pregnancy : Virulence Factors Contributing to Vaginal Colonisation. Int J Environ Res Public Health. 2014;11(7):6979–7000.
3. Osman Erkmen. Practice 10 Gram Staining Technique. In: Laboratory Practices in Microbiology. s.l.:Elsevier. 2022. p. 99-105.
4. Rahim A. Dasar Pemeriksaan Kuman-Kuman Aerob, Mikroaerofilik, dan Anaerob. In: Mikrobiologi Kedokteran. 2014. p. 70–4.
5. Samuel LP, Balada-llasat J, Harrington A. Multicenter Assessment of Gram Stain Error Rates. J Clin Microbiol. 2016;54(6):1442–7.
6. Sandle T. Assessing Gram-Stain Error Rates within The Pharmaceutical Microbiology Laboratory. Eur J Parenter Pharm Sci. 2020;25(1):1–14.
7. Jiwintarum Y, Rohmi, Prayuda IDPM. B Buah Naga (Hylocereus Polyrhizus) sebagai Pewarna Alami untuk Pewarnaan Bakteri. J Kesehat Prima. 2016;10(2):1726–34.
8. Nishant Tripathi, Amit Sapra. Gram Staining (1 ed.). Treasure Island (FL): StatPearls. 2021.
9. Lyudmila Boyanova. Direct Gram Staining and Its Various Benefits in the Diagnosis of Bacterial infections. Postgraduate Medicine. 2013;130(1):105-110.
10. Anvarinejad M, Pouladfar G, Japoni A, Bolandparvaz S, Satiary Z, Abbasi P, et al. Isolation and Antibiotic Susceptibility of the Microorganisms Isolated from Diabetic Foot Infections in Nemazee Hospital , Southern Iran. J Pathog. 2015;2015:1–7.
11. Del Rio-Pertuz G, Gutiérrez JF, Triana AJ, Molinares JL, Robledo-Solano AB, Meza JL, et al. Usefulness of sputum gram stain for etiologic diagnosis in community-acquired pneumonia: A systematic review and meta-analysis. BMC Infect Dis. 2019;19(1):1–12.
12. Bautista CT, Wurapa E, Sateren WB, Morris S, Hollingsworth B, Sanchez JL. Bacterial vaginosis : a synthesis of the literature on etiology , prevalence , risk factors , and relationship with chlamydia and gonorrhea infections. Mil Med Res [Internet]. 2016;3(4):1–10. Available from: http://dx.doi.org/10.1186/s40779-016-0074-5.
13. Martin DH, Marrazzo JM. The Vaginal Microbiome : Current Understanding and Future Directions. J Infect Dis. 2016;3(Suppl 1):1–6.
14. Chacra LA, Fenollar F, Diop K. Bacterial Vaginosis : What Do We Currently Know ? Front Cell Infect Microbiol. 2022;11(January):1–13.