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Original Article

Comparison of Methylene Blue versus Patent Blue Dyes in Sentinel Lymph Node Biopsy: A Prospective Study at A Tertiary Care Hospital

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ABSTRACT

Objective: To compare the diagnostic accuracy of Methylene blue versus patent blue dyes in the identification of sentinel lymph node (SLN) on biopsy, and compare clinical patient outcomes, including adverse effects and local tissue effects.
Study Design: Quasi-Experimental study.
Place and Duration of Study: Department of Breast Surgery, Combined Military Hospital, Rawalpindi Pakistan, from Mar to Sep 2025.
Methodology: A total 240 were divided to identify sentinel lymph node using Methylene blue (n=120) versus patent blue dyes (n=120). Primary variable studied was the diagnostic accuracy of both dyes in detection of sentinel lymph node compared with the actual histopathology report. Secondary variables studied were incidence and frequency of adverse effects between both groups.
Results: Diagnostic comparison of Methylene blue compared to actual histopathology results showed a diagnostic accuracy of 90.0% while comparison of patent blue dye showed a diagnostic accuracy of 95.8%. Frequency of adverse effects showed that hypersensitivity was seen in 05(4.2%) versus 07(5.8%) patients (p=0.769), skin rash was seen in 04(3.3%) versus 08(6.7%) patients, bronchospasm was seen in 00(0%) versus 02(1.7%) patients (p=0.498), confusion was seen in 01(0.8%) versus 03(2.5%) patients (p=0.622), dizziness was seen in 02(1.7%) versus 05(4.2%) patients (p=0.446) and nausea was seen in 02(1.7%) versus 04(3.3%) patients (p=0.684).
Conclusion: The study concludes that, combined with the radioisotope, both methods show comparable detection rates for sentinel lymph nodes with a favorable and comparable adverse effects profile.

Keywords: Breast, Biopsy, Blue, Dye, Lymph, Methylene, Node, Sentinel.

How to Cite This Article: *Javed A, Naz S, Naqvi SRQ, Saleema A, Akram Z, Naqvi SSQ. Comparison of Methylene Blue versus Patent Blue Dyes in Sentinel Lymph Node Biopsy: A Prospective Study at A Tertiary Care Hospital. Pak Armed Forces Med J 2025; 75(6): 1255-1260.*
DOI: https://doi.org/10.51253/pafmj.v75i6.13915

INTRODUCTION

Sentinel lymph node biopsy (SLNB) is now established as the gold standard for axillary staging in early breast cancer and is increasingly adopted in other malignancies.[1] Among tracers, blue dyes such as Methylene blue (MB) and patent blue dye (PBD) remain widely used, especially in settings where isotopes or fluorescence are unavailable.[2] While PBD has been widely validated, it is associated with allergic reactions and higher cost, leading to interest in MB as a safer and more economical alternative. But patient outcomes with associated adverse effects between both have not been studied extensively in our demographic setups to make conclusive diagnostic recommendations.[3] Comparative studies have evaluated diagnostic accuracy and safety, but findings vary across health systems and are shaped by local resource constraints.[4]

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Received: 04 Oct 2025; revision received: 06 Nov 2025; accepted: 12 Nov 2025

International studies show both MB and PBD achieve high detection rates, often >90% when combined with isotopes. Reviews confirm the non-inferiority of MB, though some cohorts report slightly lower identification rates compared to PBD. Locally, in South Asia and Africa, MB is widely used due to limited PBD availability, with detection accuracies reported between 85–95%.[5] However, local studies in Pakistan show smaller cohorts and single-center experiences, contrasting with the multicenter, randomized approaches in international trials.[6]

Despite promising evidence, key gaps remain. International studies increasingly focus on novel tracers and dye–isotope combinations, sidelining MB-focused research.[7] Local studies highlight MB's feasibility but lack methodological rigor, limiting generalizability. Head-to-head randomized comparisons of MB and PBD are sparse. Addressing these gaps is essential for evidence-based diagnostic recommendations, adopting a holistic method covering not only diagnostic accuracy but adverse

effect outcomes as well. The aim of this study is to compare the diagnostic accuracy of Methylene blue versus patent blue dyes in the identification of sentinel lymph node (SLN) on biopsy and compare clinical patient outcomes, including adverse effects and local tissue effects.

METHODOLOGY

This quasi-experimental study was carried out at the Department of Breast Surgery, Combined Military Hospital (CMH), Rawalpindi Pakistan, from March 10, 2025, to September 10, 2025, after approval from the ethical review board (vide letter no 828 dated Mar 10, 2025). The sample size for two groups was calculated keeping the confidence interval at 95%, power of test at 80%, with the anticipated sensitivity of Methylene blue versus patent blue dye in the detection of sentinel lymph node on biopsy at 88% versus 96% respectively.[5] Minimum sample size using the WHO calculator came out to be 217 patients. We included 240 patients in the final assessment protocol, divided into Group-A and Group-B using simple random sampling via lottery method according to the inclusion criteria furnished (Figure).

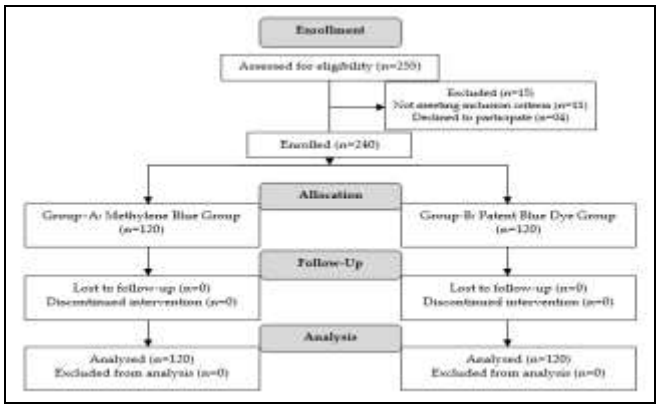

Figure: Patient Flow Diagram

Inclusion Criteria: included female patients aged 18–70 years with a histologically confirmed diagnosis of early-stage invasive breast cancer (T1–T2, N0, M0), clinically node-negative axilla on physical examination and ultrasound, and patients scheduled for breast-conserving surgery or mastectomy with sentinel lymph node biopsy.

Exclusion Criteria: included Patients with pregnancy, lactation, or a history of allergic reactions to blue dyes, those with locally advanced disease (T3–T4 or N1–N3), distant metastasis, or serious comorbid conditions precluding anesthesia or surgery, patients with prior breast or axillary surgery which can alter lymphatic drainage patterns, patients lost to follow-up or non-consent to be included in the study protocol.

The study method included all patients as per the inclusion criteria furnished. The patients were divided into two groups of 120 patients each. Group A (n=120) to receive Methylene blue as the detecting agent and Group B (n=120) to receive patent blue dye as the detecting agent. The patients were counseled in detail about the study protocol, methods of sampling, procedural complications, and the need to follow up, but were not told about the group allocation and study outcomes of the study to ensure blinding and prevent bias.

All patients were scheduled for elective surgery, and pre-anesthesia assessment for fitness was done as per protocol. Group A (n=120) underwent SLN mapping with Methylene blue (MB) injection, while Group B (n=120) received patent blue dye (PBD). In both groups, 2–5 ml of dye was injected subareolar approximately 5–10 minutes before surgery. In both groups dye was injected along with Tc-99 radioisotope injection. Surgeons performing the biopsy were blinded to allocation until the time of dye injection. The sentinel lymph node was identified intra-operatively as the first blue-stained node along the lymphatic drainage pathway and excised for histopathological evaluation. If no node was identified within 30 minutes, it was marked as failed sentinel and axillary clearance was done and excluded from final assessment in the study protocol and added to the exclusion criteria. All excised nodes underwent histopathology and subsequent hematoxylin–eosin and immunohistochemical analysis. Standardized operative checklists were maintained to reduce procedural variability.

The samples were then sent for histopathological examination, ensuring that histopathologists assessing excised nodes remained blinded to the dye used to prevent interpretation bias. All the reports were collected by a resident surgeon unaware of the study protocol and were submitted to an independent team for statistical analysis. All patients were observed per-operatively, in the recovery, and were followed up for 7 days for the incidence of adverse effects. The primary variable studied was the diagnostic accuracy of both dyes in the detection of the sentinel lymph node compared with the actual histopathology report. Secondary variables studied were the incidence and frequency of adverse effects between both groups.

Statistical data, including age and weight, were expressed as Mean±SD and compared using the independent samples t-test. Tumor type and laterality were expressed as frequency with percentage. A 2x2 table method was used to find the diagnostic accuracy of Methylene and patent blue dye compared with the actual histopathological finding along with sensitivity, specificity, positive and negative predictive values. Adverse effects including hypersensitivity, skin rash, bronchospasm, confusion, dizziness, nausea, skin necrosis were expressed as frequency with percentage and compared using the Fisher exact test. A *p*-value of ≤0.05 was considered statistically significant. All statistical calculations were performed using Statistical Package for Social Sciences 26.0.

RESULTS

A total of 240 patients were assessed in the final study assessment protocol for statistical analysis. They were divided into Group-A (n=120), comprising of the Methylene blue group and Group-B (n=120) comprising of the patent blue dye group. Mean age between both groups was 53.41±7.42 years versus 54.56±8.14 years (*p*=0.254). Mean weight was 68.10±9.10 kg versus 69.15±9.56 kg (*p*=0.382). Tumor type on histology showed that in-situ cases were seen in 20(16.7%) versus 05(4.2%) patients, microinvasive were seen in 20(16.7%) versus 20(16.7%) patients, invasive ductal carcinoma was seen in 60(50.0%) versus 68(56.7%) patients and invasive lobular carcinoma was seen in 20(16.7%) versus 27(22.5%) patients. Laterality showed right side of the breast involved in 76(63.3%) versus 74(61.7%) patients while left side involved in 44(36.7%) versus 46(38.3%) patients (Table-I).

Table-I: Demographic Characteristics between Both Groups (n=240)

Variables	Group A (n=120)	Group B (n=120)	*p*-value
Mean age (years)	53.41±7.42	54.56±8.14	0.254*
Mean weight (kg)	68.10±9.10	69.15±9.56	0.382*
Histological type			
In situ	20(16.7%)	05(4.2%)	-
Microinvasive	20(16.7%)	20(16.7%)	-
Invasive ductal	60(50.0%)	68(56.7%)	-
Invasive lobular	20(16.7%)	27(22.5%)	-
Laterality			
Right	76(63.3%)	74(61.7%)	-
Left	44(36.7%)	46(38.3%)	-

**Independent samples t-test*

Diagnostic comparison of Methylene blue compared to actual histopathology results showed a sensitivity of 91.4%, specificity of 88.7%, a positive predictive value of 88.3% and a negative predictive value of 91.7% with a diagnostic accuracy of 90.0% while comparison of patent blue dye compared to actual histopathology results showed a sensitivity of 96.6%, specificity of 95.2% with a positive predictive value of 94.9% and negative predictive value of 96.7% and diagnostic accuracy of 95.8% (Table-II).

Table-II: Diagnostic Accuracy of Methylene Blue versus Patent Blue Dye Compared to Actual Histopathology Report (n=160)

	Histopathology Findings	
	Yes/Positive	No/Negative
Sentinel Node By Methylene Blue Yes/Positive No/Negative	53(44.1%) 05(4.16%)	07(5.83%) 55(45.8%)

Sensitivity= True Positive/ (True Positive +False Negative) = 91.4%
Specificity= True Negative / (True Negative +False Positive) = 88.7%
Positive Predictive Value= True Positive/ (True Positive+ False Positive) = 88.3%
Negative Predictive Value=True Negative/ (True Negative +False Negative)=91.7%
Diagnostic Accuracy= (True Positive +True Negative)/All Patients = 90%

	Histopathology findings	
	Yes/Positive	No/Negative
Sentinel node by patent blue dye Yes/Positive No/Negative	56(46.6%) 02(1.6%)	03(2.5%) 59(49.1%)

Sensitivity= True Positive/ (True Positive +False Negative) = 96.6%
Specificity= True Negative / (True Negative +False Positive) = 95.2%
Positive Predictive Value= True Positive/ (True Positive+ False Positive) = 94.9%
Negative Predictive Value=True Negative/ (True Negative +False Negative)=96.7%
Diagnostic Accuracy= (True Positive +True Negative)/All Patients = 95.8%

Table-III: Adverse Effects Profile between Both Groups (n=160)

Variables	Group A (n=80)	Group B (n=80)	*p*-value
Hypersensitivity	05(4.2%)	07(5.8%)	0.769*
Skin rash	04(3.3%)	08(6.7%)	0.375*
Bronchospasm	00(0%)	02(1.7%)	0.498*
Confusion	01(0.8%)	03(2.5%)	0.622*
Dizziness	02(1.7%)	05(4.2%)	0.446*
Nausea	02(1.7%)	04(3.3%)	0.684*
Skin necrosis			
Mild epidermolysis	03(2.5%)	01(0.8%)	0.244*
Partial necrosis	02(1.7%)	00(0%)	
Complete necrosis	00(0%)	00(0%)	

**Fisher-exact test*

Frequency of adverse effects showed that hypersensitivity was seen in 05(4.2%) versus 07(5.8%) patients (*p*=0.769), skin rash was seen in 04(3.3%) versus 08(6.7%) patients, bronchospasm was seen in 00(0%) versus 02(1.7%) patients (*p*=0.498), confusion was seen in 01(0.8%) versus 03(2.5%) patients (*p*=0.622), dizziness was seen in 02(1.7%) versus 05(4.2%) patients (*p*=0.446) and nausea was seen in

02(1.7%) versus 04(3.3%) patients (p=0.684). Skin necrosis as mild epidermolysis was seen in 03(2.5%) versus 01(0.8%) patients, partial necrosis in 02(1.7%) versus 00(0%) patients, and complete necrosis was seen in 00(0%) versus 00(0%) patients between both groups (p=0.244) (Table-III).

DISCUSSION

Our study concluded that detection of sentinel lymph node by Methylene blue and patent blue dyes shows excellent diagnostic accuracy achieving rates more than 90% in our study. This comparable accuracy favors more towards Methylene blue due to its cost-effectivity, safety and better integration with isotopes. While patent blue dyes shows a slightly higher diagnostic accuracy, they slightly increased adverse effects profile goes to favor Methylene blue at our resource constrained setups. While the adverse effects profile is safe for both, Methylene blue shows an overall better method for SLNB detection.

Comparison and critical analysis of our study with local and international literature shows that a large single-center retrospective analysis from China by Yang *et al.,* (2023) reported that combining MBD with indocyanine green (ICG) achieved a 98.3% identification rate in early breast cancer, with histopathology confirming metastases in all detected nodes.[8] The study demonstrates that MBD, particularly in conjunction with fluorescence techniques, provides a reliable correlation between intraoperative detection and final pathology, rivaling or surpassing traditional blue dye approaches. It also shows that combining with isotopes increases the accuracy to more than 90% as seen in our study.

Demographic experiences, particularly in resource-limited settings, emphasize the utility of MBD as a standalone method. Devarakonda *et al.,* (2021) in India found SLNB with MBD alone achieved detection rates above 90%, with histopathological findings closely aligning with intraoperative localization. Importantly, this approach was cost-effective and avoided the hypersensitivity risks associated with PBD, making it highly adaptable for centers without nuclear medicine support.[9] These results support findings of our study with Methylene blue dye showing a better adverse effects profile while frequency for both method remains less than 5%. By contrast, European data highlight the enduring role of PBD. Olivier *et al.,* (2021), in a French retrospective series of 332 patients, found that PBD reliably detected sentinel nodes, with histopathology confirming micro metastases in 42 patients. However, they reported instances of false negatives on standard histology, underlining that while PBD remains effective, its performance is not infallible and carries the added concern of allergic reactions.[7] Brazilian randomized trials offer direct comparisons between PBD and MBD. Paulinelli *et al.,* (2017), though slightly beyond the five-year window, remains influential in showing equivalent detection rates between MBD and PBD, with both dyes identifying sentinel nodes later confirmed as metastatic on histopathology.[10] The study reinforced MBD as a safe and effective alternative, particularly where PBD availability is limited. This can be applied to our local centers as well where cost remains a major issue.

Budner *et al.,* (2022) evaluated near-infrared fluorescence-guided MBD in Poland and concluded that this technique enhanced intraoperative visualization while maintaining high concordance with final pathology. Compared with PBD, MBD demonstrated fewer adverse reactions and more versatile integration with imaging technologies, suggesting it may gradually replace PBD in technologically advanced centers.[11] Comparative pathology-focused studies from Indonesia also strengthen the case for MBD. Hermansyah *et al.,* (2021) reported that despite limited access to PBD, MBD alone maintained strong accuracy in SLNB, with histopathological grade III tumors showing consistent dye-pathology correlation.[12] This highlights MBD's role in diverse tumor biology contexts without sacrificing diagnostic precision.

Local literature shows that Siddique *et al.,* (2020) compared the hybrid Tc-99m scintigraphy method with blue dye (including Methylene blue and patent blue alternatives) for SLNB in early breast cancer and reported that blue dye methods alone had lower identification rates compared to hybrid scintigraphy, but Methylene blue remained an accessible and safe tracer in local practice.[13] This is in line with results of our study where isotope addition increased the reported accuracy to more than 90%. Saleem *et al.,* (2025), investigated factors influencing SLNB identification when using Methylene blue dye. It concluded that tumor size, location, and surgeon experience influenced detection rates, but overall, MBD was effective and provided strong correlation with histopathology for node-positive disease.[14] Akbar *et al.,* (2020) describes the early experience of establishing SLNB services in a Pakistani public

hospital, where Methylene blue dye was used as the primary tracer due to the unavailability of patent blue dye. Results showed reliable identification of sentinel lymph nodes in clinically axilla-negative breast cancer patients, with histopathology confirming the accuracy of MBD mapping.[15] This goes in line with our recommendations for low resource countries and setup where availability of patent blue dyes is also an issues and with comparative results for detection, Methylene blue remains superior.

In comparative studies, Donigiewicz *et al.,* (2023) questioned the necessity of blue dyes like PBD in dual localization. Their study found that omitting PBD did not compromise histopathological outcomes, as all positive sentinel nodes were still detected with other methods. This suggests a gradual decline in reliance on PBD in favor of MBD or isotopic tracers, reflecting evolving surgical practices.[16] Xu *et al.,* (2022) explored the dual use of indocyanine green (ICG) with MBD in Chinese patients undergoing SLNB. The study concluded that the combination improved sentinel node detection while maintaining high correlation with histopathological findings. In comparison, reliance on PBD alone was less effective, showing the growing preference for MBD-based multimodal approaches.[17] Jameel *et al.,* (2024) compared PBD with technetium-99m colloid in a cross-sectional study involving breast cancer patients. While PBD performed adequately for SLNB, the combination with radiotracer improved histopathological correlation and reduced false negatives. This study suggests that although PBD has diagnostic value, its stand-alone role is increasingly questioned compared with MBD-based or hybrid methods.[18]

LIMITATION OF STUDY

Both Methylene blue dye (MBD) and patent blue dye (PBD) carry important limitations despite their clinical utility in sentinel lymph node biopsy (SLNB). MBD, while inexpensive and widely available, may have lower staining intensity than PBD, leading to occasional difficulties in identifying nodes, particularly in obese patients or those with deep axillae. It also has a slightly higher false-negative rate when used alone compared to isotope or dual methods. PBD, on the other hand, provides vivid staining but is associated with rare yet potentially severe allergic reactions, including anaphylaxis, which limit its safety profile. Additionally, PBD is more costly and less accessible.

CONCLUSION

The study concludes that combined with isotope, both methods show comparable detection rates for sentinel lymph nodes with a favorable and comparable adverse effects profile.

Conflict of Interest: None.

Funding Source: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

AJ & SN: Data acquisition, data analysis, critical review, approval of the final version to be published.

SRQN & AS: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

ZA & SSQN: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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