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RESEARCH ARTICLE
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FROZEN SECTION UNDER MICROSCOPE: AUDITING DIAGNOSTIC PITFALLS IN INTRAOPERATIVEPATHOLOGY

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Abstract:

Background: Sometimes there is a disagreement between the frozen section (FS) and the histopathological diagnosis because of significant limitations in FS processing and reporting, such as poor morphology and freezing artifact. A medical audit of FS reports was conducted to identify the cause of diagnostic limitations in FS reporting and formulating preventive measures to overcome these limitations.

Objectives: To identify the cause of diagnostic limitations in FS reporting and formulating preventive measures to overcome these limitations

Material and Methods: This retrospective medical audit done in the Department of Pathology in a tertiary hospital. The study was done from January 2024 to June 2024. The records of 48 FS and the corresponding final formalin fixed paraffin embedded (FFPE) histopathology reports during the study period of 6 months were compared and analyzed. The results were categorized into concordant, discordant. The slides of the discordant cases were reviewed and the causes of discrepancy were noted.

Results: 70% FS referral were by Surgical oncology team. Percentage of discordance was seen in 8.6 % cases (4 cases out of 48). 94.5% FS consultations had average turnaround time of 25 min. In the remaining cases (5.5%) the turnaround time (TAT) was around 30-35 min.

Conclusion: FS is a valuable intraoperative diagnostic tool with high concordance to final histopathology, supporting critical surgical decisions. While accuracy may be affected by sampling, technical, and interpretative factors, most discrepancies are preventable through standardization, quality control, and ongoing education. Targeted training for surgeons and continuous auditing are essential to maintain diagnostic accuracy, enhance patient safety, and optimize frozen section services.

Keywords: Frozen section; discordant; audit, intraoperative pathology

Introduction:

The frozen section (FS) technique was initially introduced by De Riemer in 1818 (1). A thin slice of frozen tissue, known as a frozen section (FS)**,** serves as a rapid intraoperative diagnostic specimen. In 1949, Hazard and Stevenson (2) refined the method by fixing tissues in a heated fixative, freezing them with dry ice, sectioning, staining, and mounting the slides. The advent of cryostat technology further enhanced FS by enabling the preparation of thin sections (5–10 μm) and the use of modified H&E staining for permanent slides. A worldwide survey of 700 laboratories found that 90% of FS diagnoses were reported within 20 minutes of receiving the specimen (3). Surgeons rely on FS results to guide the extent of the procedure. However, due to its rapid preparation and limited sampling, the FS is not suitable for a detailed cellular examination**.** Minimizing discrepancies between FS diagnoses and the final pathology report requires a deep understanding of pathology and laboratory procedures. To ensure the accuracy of FS reporting, pathologists engage in quality assurance practices**.** Addressing the inherent limitations of FS involves identifying the causes of diagnostic errors and implementing preventative measures. By understanding these constraints and refining laboratory protocols, diagnostic reliability and patient care during surgery can be significantly improved

The pathologist ensures accurate FS by reviewing relevant tumor information, prior slides, and clinical data. During FS, they provide only essential guidance—confirming lesional tissue or distinguishing benign from malignant—while detailed grading is rarely needed. They also decide whether FS is necessary, balancing intraoperative support with patient safety to avoid premature or incorrect diagnoses.

Results:

The pie chart titled Different Departments that had FS Consultations (Figure 1) illustrates the proportion of frozen section cases received from various hospital departments. The majority of consultations, accounting for 70%, originated from the Surgical Oncology department, indicating that most intraoperative diagnostic requests are related to cancer surgeries where margin assessment and rapid diagnosis are crucial. Gynecology contributed 15% of the cases, reflecting its regular use of frozen sections for evaluating ovarian, uterine, and cervical lesions. The ENT department accounted for 10%, primarily for head and neck tumor evaluations, while other departments represented 5%, indicating occasional utilization of the service for diverse surgical needs.

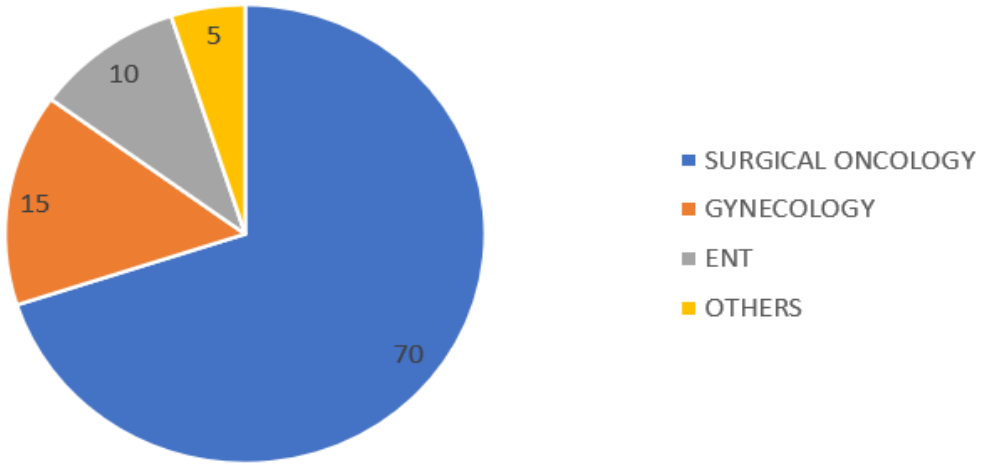


Figure 1: Distribution of frozen section consultations among different departments.

The pie chart titled Concordance and Discordance Rates (Figure 2) illustrates the comparison between cases where the frozen section diagnosis matched the final histopathology report (concordant) and those where it did not (discordant). The chart shows that 92% of the cases were concordant, indicating a high level of accuracy and reliability of frozen section diagnoses in the laboratory. Only 8% of the cases were discordant, representing minor discrepancies between the intraoperative and final reports. This distribution demonstrates that the FS technique provides a dependable diagnostic tool for intraoperative consultation, aiding surgeons in making immediate clinical decisions. The small proportion of discordant cases may be attributed to factors such as sampling errors, tissue artifacts, or interpretational challenges, which are inevitable in certain situations. Overall, the chart reflects excellent diagnostic concordance, signifying the effectiveness and consistency of frozen section procedures in the pathology laboratory.

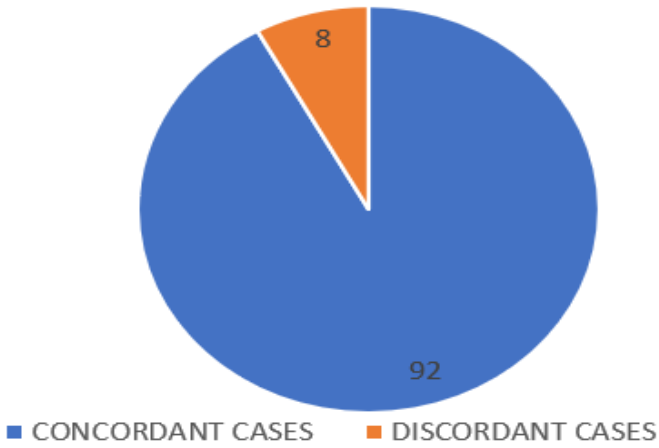
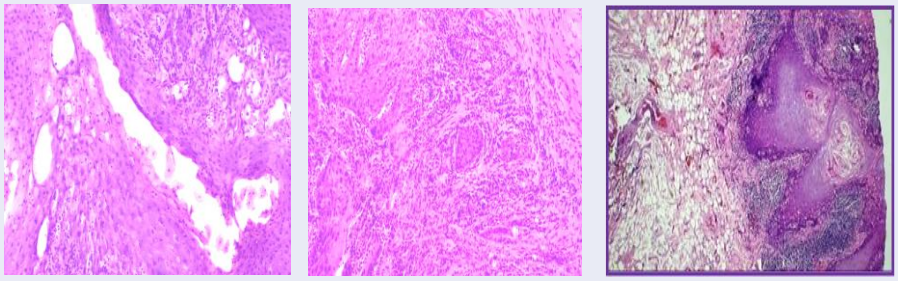
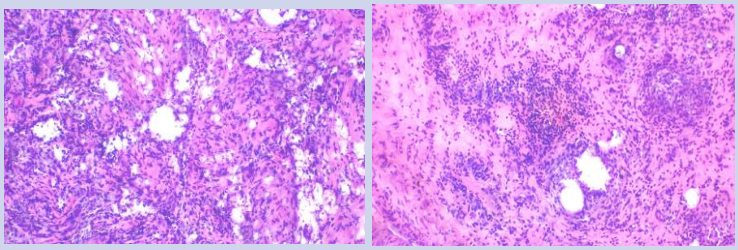
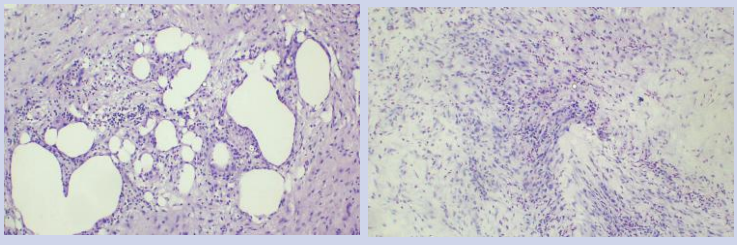
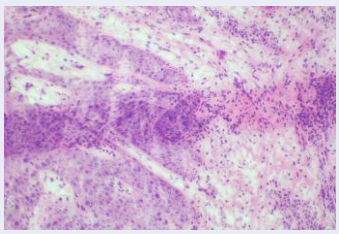
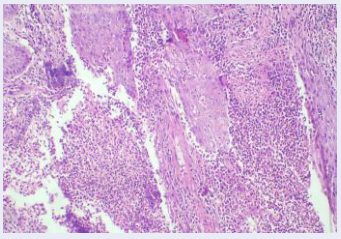


Figure 2: Concordance and discordance rates between frozen section and final histopathology diagnoses.

Number	Site	Frozen diagnosis	HPE diagnosis	Cause of discrepancy

CASE 1-06/2024	Buccal mucosa	High grade dysplasia	Exophytic squamous cell carcinoma	Sometimes invasion is seen only on extensive grossing done later on, tumor present only on deeper cuts
CASE 2-30/2024	Buccal mucosa	SIN 3 with suspicion of invasion	SIN3 (With no invasion.)	Tangential cutting of tissue.
				Fig 2a, show's irregular nests of atypical epithelial cells with crowded, pleomorphic nuclei; 2b: shows a densely cellular, disorganized spindle-cell proliferation consistent with a stromal or mesenchymal lesion; 2c: keratin pearl formation surrounded by atypical squamous cells, indicative of well-differentiated squamous cell carcinoma.
CASE3-20/2024	Omental deposits for recto sigmoid tumor	Suspicious of adenocarcinoma	Negative for malignancy	Holes in the tissue, dark nucleus
				Fig 3a/3b : H&E-stained tissue section showing granulomatous inflammation with scattered necrotic, pale acellular areas
				Fig 3c, 3d shows H&E-stained section showing cystic, variably sized empty spaces consistent with fat necrosis surrounded by inflammatory cells, A low-power microscopic view showing loosely arranged spindle-shaped cells with a whorled pattern, consistent with a fibrous or stromal lesion.
CASE4-27/2024	Buccal mucosa	Atypical squamous proliferation	SIN2 with focal invasion	Patchy staining, sometimes invasion is seen on deeper cuts only, Processing limitation-folds within

				the histological section due to blunt microtome.
 				Fig 4a, 4b shows A microscopic image showing dense inflammatory cell infiltrates with areas of necrosis and surrounding fibrous stroma, sheets of densely packed small round cells with scant cytoplasm separated by thin fibrous septa.

Discussion : The FS, also known as the cryostat section, is a rapid intraoperative diagnostic procedure primarily used to determine whether a tissue sample is benign or malignant. During surgery, it provides critical information that assists the surgeon in assessing the extent of disease involvement and deciding the scope of surgical excision. For instance, if a margin is reported as positive for malignancy on frozen section, the surgeon may immediately extend the surgical margins. Additionally, FS examination plays a potential role in identifying suspected metastases; confirmation of metastatic deposits enables the surgeon to modify or extend the operative plan accordingly.
The procedure involves freezing the tissue, during which the water content in the specimen solidifies into ice**,** providing firmness and allowing ice to serve as the embedding medium. The typical workflow includes gross examination of the specimen followed by sectioning using a cryostat. Thin sections, usually around 4 microns in thickness, are prepared, stained, and examined microscopically by a senior, experienced pathologist. The preliminary report is then promptly communicated to the operating surgeon to guide intraoperative decision-making. Following the FS diagnosis, the remaining tissue and frozen fragments are fixed in 10% neutral buffered formalin overnight and subsequently processed through the routine histopathology workflow. The final histopathology report is issued after detailed examination and confirmation by senior pathologists on permanent paraffin-embedded sections.

The limitations of FS fall into three main categories: sampling errors, where the tissue may be inadequate or unrepresentative; technical challenges, including difficulties with freezing, cutting, or staining the tissue; and interpretative difficulties, where subtle or complex histologic features may lead to diagnostic uncertainty. These factors highlight that, while FS is a valuable intraoperative tool, it has inherent constraints that can affect accuracy (4,5)

1	Poor sampling of tissue
2	Poor selection of appropriate tissue after grossing
3	Extensive tumor degeneration
4	Poor assessment of capsular or vascular invasion
5	Freezing artefacts
6	Poor quality section
7	Bloated cell morphology
8	Poorly stained section
9	Interpretive errors due to heterogeneity of tumor, biphasic tumor, variable degree of tumor heterogeneity.

Table 1: Limitations while performing Frozen section (FS)

In the present study, a total of 44 cases (92%) were concordant and 4 cases (8%) were discordant when comparing FS diagnoses with final histopathology results. The observed discordance rate falls

within the range reported in various studies, which varies from 1.4% to 12.9% depending on the anatomical site examined, indicating that our findings are comparable to previously published data. The main causes of discordance in our study were technical limitations and interpretation errors, with the latter primarily resulting from limited tissue sampling and the presence of invasion only in deeper tissue levels.

Historical data also support these findings. As early as 1985, Dankwa et al. reported a large series of 1000 frozen sections, achieving a 96.5% accuracy rate, with 1.3% of cases showing clinically significant errors (6). The causes of inaccuracy were attributed to the localized nature of lesions (14 cases), pathological misinterpretation (28 cases), and technical imperfections (3 cases). They noted that misinterpretation accounted for all avoidable diagnostic errors. Similarly, Selvakumar et al., in a retrospective study of 518 specimens, documented an accuracy rate of 98.65% and an error rate of 1.35% (7). The oropharyngolaryngeal region (48.84%) constituted the majority of samples, with margin status evaluation (52.27%) being the most common indication for frozen section analysis. In their study, discordance mainly resulted from sampling errors and misinterpretation. Furthermore, Sekhar et al. emphasized in their retrospective review that understanding the causes and pitfalls of diagnostic discrepancies is essential for improving frozen section accuracy. Their study also identified interpretation errors and inadequate sampling as the predominant factors contributing to discordant results. A retrospective study by Tangde et al. (8) evaluated FS accuracy by comparing intraoperative diagnoses with final permanent sections. Among 83 patients (73 females, 10 males), 76 FS diagnoses were concordant, and seven were discordant, yielding an accuracy of 91.57%, sensitivity 85.71%, specificity 97.92%, positive predictive value 96.77%, and negative predictive value 90.38%. FS correlation with permanent sections is essential for quality assurance. A review of 1,207 frozen sections from 812 cases at Hamilton Regional Laboratory Medicine Programme (2007) found 24 discordant diagnoses (3%), mainly due to misinterpretation or sampling errors. Frozen sections were most common for head and neck, nervous system, and female genital tract specimens, with potential adverse clinical impact in 14 cases (9).

Another retrospective review by Hatami et al. evaluated frozen sections performed at Taleghani Hospital, Tehran, Iran (2007–2013), comparing them with permanent sections to assess accuracy, sensitivity, and specificity. Discordant cases were analyzed to identify reasons for discrepancies. Frozen section proved to be an accurate and valuable diagnostic tool for surgical management, with results comparable to internationally reported rates (10).

The literature reports discordance rates for frozen section diagnoses ranging from 1.4% to 12.9% across different anatomical sites (11,12).

NUMBER	CAUSE OF DISCREPANCY	EVITABLE OR INEVITABLE
1	Patchy staining	Evitable
2	Dark nucleus	Evitable
3	Limited sampling	Inevitable
4	Tangential cutting	Evitable
5	Holes in the tissue	Evitable

Table 2: The table outlines the common causes of discrepancies encountered during histopathological and cryosection procedures. It categorizes each issue as evitable (preventable) or inevitable (unavoidable)

The corrective and preventive measures undertaken in the laboratory are aimed at maintaining accuracy, reliability, and overall quality in frozen section diagnostic reporting. These actions were formulated after identifying the common causes of technical and interpretational discrepancies observed during frozen section processing.

Patchy staining is a common evitable problem in both frozen and paraffin sections. It usually occurs due to variations in section thickness, uneven spreading of the section, improper cryostat knife temperature, or contamination with residual cryocompound. Such irregularities result in uneven stain absorption and make microscopic interpretation difficult (13). To rectify this, laboratory personnel ensure uniform section thickness, verify knife temperature and sharpness, check the tightness of the knife clamp, and remove any remaining cryocompound from the disc. Maintaining optimal cutting conditions greatly improves the quality and uniformity of staining.

Another frequent issue is the presence of dark nuclei due to overstaining with hematoxylin. This evitable discrepancy often results from prolonged staining time or the use of overly concentrated staining solutions. To prevent this, the hematoxylin staining time is reduced from five minutes to one minute, and regular calibration of staining solutions is carried out. Consistent timing during staining ensures reproducibility and clarity of nuclear features, thereby improving diagnostic precision.
Limited sampling, on the other hand, is an inevitable limitation in frozen section procedures. It occurs when only a small portion of tissue is available for examination or when time constraints during intraoperative consultations restrict sampling depth. Even with experienced personnel, this may lead to partial lesion representation or missing critical pathological areas. The laboratory addresses this limitation by documenting sampling constraints, obtaining deeper sections when possible, and maintaining close communication with the surgical team to ensure representative sampling.

Tangential cutting is another evitable cause of discrepancy that leads to distortion of tissue architecture and misinterpretation of tissue layers. It occurs when the cutting angle is incorrect or the tissue block is not properly aligned. To correct this, the angle of the cutting blade is checked and adjusted to ensure perpendicular sectioning. Proper orientation of the tissue block and regular maintenance of the cryostat or microtome are essential to prevent such errors.
Holes in tissue sections are often caused by overfreezing, especially in edematous tissues. Overfreezing leads to ice crystal formation, which disrupts tissue integrity and creates artifacts that compromise diagnostic accuracy. To minimize this problem, the freezing temperature is carefully monitored, and overfreezing is avoided. Using optimal cryostat settings and proper embedding techniques helps maintain structural preservation.

These findings emphasize the importance of strict quality control, preventive maintenance, and continuous staff training in histopathology laboratories. While some discrepancies like limited sampling may be unavoidable due to external constraints, most technical errors can be minimized through standardized protocols, regular equipment calibration, and meticulous handling techniques. Continuous monitoring and internal audits are essential to maintain consistency, accuracy, and diagnostic reliability.

The authors Weiss et al (14) aimed to evaluate the knowledge of surgical faculty regarding FS consultations at a university hospital and determine if this knowledge correlated with appropriateness of frozen section requests**.** The authors interpreted that although most FS requests were appropriate, there are important knowledge gaps among surgical faculty regarding FS technique and indications**.** They concluded that inappropriate requests could be reduced by targeted education for the few responsible faculty, while general knowledge should be improved broadly through case-based clinical teachingThe present study enabled the identification of both the capabilities and limitations of frozen section procedures in our laboratory. The insights gained were valuable in providing feedback and training to laboratory technicians who, along with pathologists, play a crucial role in the successful execution of frozen section diagnostics. An ongoing audit in our department continues to evaluate the effectiveness of the implemented rectifications and their role in reducing the discrepancy rate, ensuring continuous improvement in diagnostic standards and patient care.

Conclusions:

FS is a reliable and valuable intraoperative diagnostic tool that guides surgical decision-making, including margin assessment and detection of metastases. While the procedure demonstrates high concordance with final histopathology, its accuracy can be affected by sampling errors, technical challenges, and interpretative difficulties. Most discrepancies are preventable through standardized protocols, quality control, and ongoing staff training, though some, like limited sampling, are unavoidable. Targeted education for surgical faculty can reduce inappropriate FS requests, while broad case-based teaching improves general knowledge. Continuous audits and corrective measures are essential to maintain diagnostic accuracy, ensure patient safety, and optimize the effectiveness of frozen section services.

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