

Role of Special Stains in the Diagnosis of Infectious Diseases in Tissue Sections: A Retrospective Observational Study.

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Page | 1

Abstract

Background:

Histopathological examination remains an important diagnostic modality for infectious diseases, particularly when microbiological culture or molecular testing is unavailable or inconclusive. Although routine hematoxylin and eosin (H&E) staining provides important tissue information, special histochemical stains improve the visualization and identification of microorganisms in tissue sections.

Methodology:

This retrospective observational study included 350 tissue specimens received in the Department of Pathology, Government Medical College, Alwar, over two years. H&E sections were reviewed, followed by Ziehl–Neelsen (ZN), Periodic Acid-Schiff (PAS), Grocott Methenamine Silver (GMS), Gram, Fite-Faraco, Giemsa, and Mucicarmine stains whenever indicated. Clinicopathological details, organisms detected, and diagnostic yield were analyzed using descriptive statistics and the Chi-square test.

Results:

The mean age of patients was 43.8 ± 17.6 years, with the majority belonging to the 41–60-year age group (38.3%). Infectious organisms were demonstrated in 232 (66.3%) specimens. Fungal infections were the most common diagnosis (35.4%), followed by tuberculosis (31.4%). PAS and GMS showed the highest positivity for fungal infections, while ZN and Ziehl–Neelsen effectively demonstrated acid-fast bacilli. Special stains significantly improved etiological diagnosis compared with H&E alone ($p < 0.001$).

Conclusion:

Special histochemical stains remain valuable adjuncts to routine histopathology and enhance the detection and identification of infectious organisms in tissue sections.

Recommendation:

Appropriate special stains should be selected according to histomorphological findings and clinical suspicion, with correlation with microbiological and molecular methods whenever available.

Keywords: Histopathology, Special stains, *Periodic Acid-Schiff*, *Grocott Methenamine Silver*, *Ziehl–Neelsen stain*, *Infectious diseases*, *Tissue biopsy*, *Fungal infection*.

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Introduction

Despite significant advancements in microbiological diagnoses and antibiotic therapy, infectious diseases remain one of the world's top causes of morbidity and mortality. In

many infectious disorders, tissue biopsy is still a crucial diagnostic procedure, especially if the lesion is inaccessible for direct sampling, the pathogen burden is low, or microbiological cultures are negative. In addition to

identifying bacteria, histopathological examination offers important details about tissue response, illness severity, and differential diagnosis. The foundation of tissue diagnosis is routine hematoxylin and eosin (H&E) staining (1).

However, due to their tiny size, low tissue burden, masking by inflammatory exudates, or poor staining properties, microorganisms may be challenging to see on H&E. As a result, histochemical special stains are now essential tools in surgical pathology. By taking advantage of variations in the chemical makeup or composition of cell walls, certain stains highlight microorganisms in a targeted manner. Polysaccharide-rich fungal cell walls and some parasites can be seen with the Periodic Acid-Schiff (PAS) stain. For fungi like *Candida*, *Aspergillus*, *Histoplasma*, *Pneumocystis jirovecii*, and *Cryptococcus*, Grocott Methenamine Silver (GMS) stain offers superior contrast. While Fite-Faraco stain is especially helpful for *Mycobacterium leprae* and partially acid-fast organisms, Ziehl-Neelsen (ZN) stain is still the standard technique for identifying acid-fast bacilli. While Giemsa stain shows intracellular organisms like *Leishmania* and *Helicobacter* species, Gram stain makes it easier to identify bacteria. The polysaccharide capsule of *Cryptococcus neoformans* is highlighted by mucicarmine stain (2).

Even while molecular approaches like PCR, next-generation sequencing, and immunohistochemistry have transformed the diagnosis of infectious diseases, they are costly, require complex laboratory equipment, and are not available in many environments with low resources. Because they are cheap, quick, repeatable, and compatible with formalin-fixed paraffin-embedded tissues, special stains continue to be essential in standard pathology labs. According to recent research, Ziehl-Neelsen and Fite stains are still very useful for diagnosing mycobacterial illnesses, while PAS and GMS have a high specificity for fungal infections. However, organism load, tissue type, host immune condition, and adequate sampling all affect how sensitive they are (3).

The use of specific stains in conjunction with histomorphology greatly increases diagnostic confidence and directs further molecular or microbiological research. Deep fungal infections, parasitic disorders, tuberculosis, and opportunistic infections linked to diabetes, cancer, transplantation, and immunosuppression are all quite common in India. By facilitating the timely start of targeted antimicrobial therapy, early tissue diagnosis with suitable histochemical stains can significantly impact patient management (4).

Comprehensive information about the diagnostic efficacy and usage trends of special stains in routine histopathology from North Indian tertiary care facilities is still scarce,

despite their extensive use. In order to assess the effectiveness of several special stains in the diagnosis of infectious diseases in tissue sections obtained at Government Medical College, Alwar during a two-year period, the current retrospective study was conducted (5). **Therefore, this retrospective observational study aimed to evaluate the diagnostic utility of special histochemical stains in detecting infectious organisms in tissue sections and to determine their contribution to routine histopathological diagnosis at Government Medical College, Alwar, Rajasthan.**

Materials and Methods

Study Design and Setting

This **retrospective observational study** was conducted in the Department of Pathology, Government Medical College, Alwar, Rajasthan, from January 2023 to December 2024. The study involved a retrospective review of archived histopathology records, tissue specimens, and corresponding hematoxylin and eosin (H&E) and special-stain slides of cases suspected of having an infectious etiology. The study design involved identification of eligible cases from departmental records, review of available clinicopathological information and histomorphological findings, assessment of appropriate special stains, and evaluation of the organisms identified and their diagnostic yield. No additional specimens or interventions were performed specifically for the study.

Government Medical College, Alwar, is a tertiary healthcare teaching institution providing diagnostic and clinical services to patients from Alwar and surrounding areas. The institution provides outpatient and inpatient medical services along with specialized clinical services and laboratory-based diagnostic facilities, including histopathological examination, cytological evaluation, microbiological investigations, and other pathology services. The Department of Pathology provides routine H&E-based histopathological examination and special histochemical staining for the evaluation of tissue specimens received from various clinical departments.

The study was undertaken to evaluate the role of special histochemical stains in the diagnosis of infectious diseases in tissue sections and to assess their contribution to routine histopathological diagnosis.

Study Population

A total of 350 tissue specimens with histopathological suspicion of infectious etiology were included. Cases were

retrieved from the departmental archives and histopathology records.

Inclusion Criteria

- Tissue biopsies and surgical specimens with suspected infectious pathology.
- Availability of formalin-fixed paraffin-embedded tissue blocks and corresponding H&E and special stain slides.
- Adequate clinical and histopathological records.

Exclusion Criteria

- Poorly preserved or inadequate tissue specimens.
- Cases lacking special stain results.
- Repeat biopsies from the same patient.
- Autolyzed or technically unsatisfactory sections.

Histopathological Evaluation

All archived H&E-stained slides were independently reviewed by two experienced pathologists. Based on the histomorphological findings, appropriate special stains were evaluated.

The special stains included:

- Ziehl-Neelsen (ZN) stain for acid-fast bacilli
- Fite-Faraco stain for *Mycobacterium leprae*
- Periodic Acid-Schiff (PAS) stain for fungi and certain parasites
- Grocott Methenamine Silver (GMS) stain for fungal organisms
- Gram stain for bacterial infections
- Giemsa stain for protozoal and intracellular organisms
- Mucicarmine stain for encapsulated fungi, particularly *Cryptococcus neoformans*

Histopathological diagnosis was established based on tissue morphology combined with special stain findings.

Data Collection

The following variables were recorded:

- Age
- Gender
- Tissue site
- Histopathological diagnosis
- Type of inflammatory response
- Organism identified
- Type of special stain used
- Special stain positivity
- Final diagnosis

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 26.0.

Continuous variables were expressed as mean $\pm$ standard deviation, whereas categorical variables were presented as frequencies and percentages.

Associations between categorical variables were analyzed using the Chi-square test or Fisher's exact test whenever appropriate. A p-value <0.05 was considered statistically significant.

Results

Clinicodemographic Profile

A total of 350 tissue specimens were analyzed. The mean age of patients was 43.8 ± 17.6 years (range: 5–84 years). The majority belonged to the 41–60-year age group (38.3%). Males constituted 56.6% (198/350), while females accounted for 43.4% (152/350).

Table 1. Demographic Characteristics

Variable	Number	Percentage
Male	198	56.6
Female	152	43.4
Age ≤ 20 years	38	10.9
21–40 years	102	29.1
41–60 years	134	38.3
>60 years	76	21.7

Distribution of Tissue Specimens

Lymph node biopsies were the most common specimens (28.6%), followed by skin biopsies (20.6%), lung tissue (14.6%), gastrointestinal biopsies (12.9%), soft tissue (11.4%), bone (6.3%), and miscellaneous organs (5.7%).

Table 2. Distribution of Tissue Samples

Tissue	n	%
Lymph node	100	28.6
Skin	72	20.6
Lung	51	14.6
Gastrointestinal tract	45	12.9
Soft tissue	40	11.4
Bone	22	6.3
Others	20	5.7

Spectrum of Infectious Diseases

Among 350 cases, fungal infections were the most common diagnosis (35.4%), followed by tuberculosis (31.4%), bacterial infections (14.6%), leprosy (8.6%), parasitic infections (4.9%), cryptococcosis (2.9%), and mixed infections (2.3%).

Table 3. Histopathological Diagnosis

Diagnosis	n	%
Fungal infections	124	35.4
Tuberculosis	110	31.4
Bacterial infections	51	14.6
Leprosy	30	8.6
Parasitic infections	17	4.9
Cryptococcosis	10	2.9
Mixed infections	8	2.3

Utilization of Special Stains

PAS stain was the most frequently employed stain (34.3%), followed by Ziehl–Neelsen (31.4%), GMS (17.1%), Gram stain (8.6%), Fite-Faraco (5.7%), Giemsa (2.3%), and Mucicarmine (0.6%).

Table 4. Special Stains Used

Stain	Cases
PAS	120
Ziehl–Neelsen	110
GMS	60
Gram	30
Fite-Faraco	20
Giemsa	8
Mucicarmine	2

Diagnostic Yield

Special stains confirmed infectious organisms in 232 cases (66.3%). PAS and GMS demonstrated the highest positivity among fungal infections, whereas Ziehl–Neelsen was highly effective in tuberculosis. Fite-Faraco accurately demonstrated lepra bacilli in leprosy cases.

Table 5. Diagnostic Yield

Finding	Cases	%
Organism demonstrated	232	66.3
Organism not demonstrated	118	33.7

Incremental Diagnostic Utility

Routine H&E alone suggested infection in 188 cases (53.7%). Following application of special stains, definitive etiological diagnosis increased to 232 cases (66.3%), representing a statistically significant improvement ($\chi^2 = 12.84$, $p < 0.001$).

Table 6. Comparison of H&E and Special Stains

Method	Positive Diagnosis
H&E alone	188
H&E + Special stains	232

p < 0.001

Association Between Organism Type and Special Stain

A highly significant association was observed between infective organism and corresponding special stain ($\chi^2 = 168.4$, $p < 0.001$), confirming that appropriate stain selection significantly enhanced organism detection.

Discussion

In 350 tissue samples with suspected infectious illnesses, the current retrospective investigation assessed the diagnostic value of specific histochemical stains. The results show that specific stains continue to be an essential addition to standard histopathology and greatly increase diagnostic precision, especially in cases of fungal and mycobacterial diseases. The majority of patients were middle-aged, with a small male predominance. These results are consistent with earlier research, which shows that males in developing nations are more likely to have chronic infections and tuberculosis due to increased work exposure (6).

The greatest diagnostic category in our series was fungal infections. This finding is consistent with recent findings of a rise in opportunistic fungal infections caused by immunosuppression, diabetes mellitus, cancer, corticosteroid therapy, and organ transplantation. Excellent sensitivity for fungus identification was demonstrated using PAS and GMS stains. While PAS successfully illustrated fungal polysaccharide-rich cell walls, GMS offered improved contrast by emphasising fungal walls as black structures against a pale green backdrop. The second most prevalent infection was tuberculosis. Although sensitivity was reduced in paucibacillary specimens, Ziehl-Neelsen stain effectively revealed acid-fast bacilli in the majority of granulomatous lesions. Caseating granulomas and other histomorphological characteristics continued to provide useful diagnostic hints, highlighting the need for microscopic interpretation to combine tissue morphology with special stain results (7).

While Gram stain helped diagnose some bacterial diseases where organisms were hard to see on normal sections, Fite-

Faraco stain was very helpful in leprosy cases, particularly those with multibacillary disease. While Mucicarmin was useful for verifying encapsulated *Cryptococcus* species, Giemsa made it easier to identify intracellular bacteria and parasitic organisms (8). When compared to H&E alone, special stains greatly enhanced the diagnostic yield and showed microorganisms in about two-thirds of instances. These results support earlier research demonstrating that histochemical stains offer quick and economical confirmation of infectious aetiologies, particularly in situations with low resources where sophisticated molecular diagnostics might not be easily accessible (9).

Even while molecular methods like next-generation sequencing, in situ hybridization, and polymerase chain reaction provide greater analytical sensitivity, they are more expensive and require specialised equipment. Histochemical stains are still widely accessible, reasonably priced, and able to provide instantaneous morphological correlation. As a result, they still play a crucial part in standard diagnostic pathology (10). The comparatively high sample size and evaluation of several special stains across a wide range of infectious disorders are this study's main advantages. However, significant drawbacks include the retrospective design, the lack of molecular linkage, and the absence of microbiological confirmation in all cases. Future prospective investigations including molecular assays, immunohistochemistry, and culture would further clarify each particular stain's diagnostic efficacy (11).

Overall, our results demonstrate the ongoing clinical relevance of specific histochemical stains despite advancements in molecular pathology and justify their continued incorporation into standard diagnostic algorithms for infectious illnesses (12).

Conclusion

In order to diagnose infectious disorders by surgical pathology, special histochemical stains are still crucial. Special stains greatly increased organism detection and the diagnostic accuracy of routine histopathological examination in this two-year retrospective investigation including 350 tissue specimens. Ziehl-Neelsen and Fite-Faraco stains continued to be useful for diagnosing mycobacterial disorders, although PAS and GMS stains were especially useful for fungal infections. The identification of certain bacterial, parasitic, and fungal diseases was further aided by the use of Gramme, Giemsa, and Mucicarmine stains.

Special stains continue to offer quick, affordable, and accurate identification of infectious pathogens with good clinicopathological association despite the growing availability of molecular diagnostic tools. In environments with limited resources and sophisticated diagnostic facilities, their regular and prudent use is especially beneficial. To optimise diagnostic output, histopathologists should choose specific stains based on the underlying tissue reaction and clinical suspicion. The most accurate method for detecting infectious disorders in tissue sections is to integrate histomorphology, specific stains, microbiological findings, and molecular techniques when available. This allows for the prompt administration of the proper antimicrobial therapy.

Recommendation

The results of this study suggest that all tissue specimens with suspected infectious aetiology should routinely undergo specific histochemical stains, especially if normal hematoxylin and eosin (H&E) staining is not conclusive. Organism identification and diagnostic accuracy can be greatly increased by choosing the right stains based on the histomorphological pattern and clinical suspicion. To guarantee uniformity and quality, pathology labs should adopt standardised procedures for the application and interpretation of specific stains. It is advised that pathologists, microbiologists, and doctors work closely together to enable precise diagnosis and prompt start of targeted antimicrobial therapy. To improve diagnostic accuracy, special stain results should, whenever possible, be correlated with microbiological culture, immunohistochemistry, and molecular diagnostic methods. To ensure technical excellence, laboratory staff should get regular training, and periodic quality assurance programmes should be implemented. It is advised that future prospective multicenter studies that include molecular confirmation be

conducted in order to demonstrate the diagnostic efficacy and economic viability of certain histochemical stains in infectious pathology.

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List of abbreviations

- H&E – Hematoxylin and Eosin
- ZN – Ziehl-Neelsen
- PAS – Periodic Acid-Schiff
- FFPE – Formalin-Fixed Paraffin-Embedded
- GMS – Grocott Methenamine Silver
- PCR – Polymerase Chain Reaction
- SD – Standard Deviation

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Author Biography

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Author Contributions

Kamna Chaudhary contributed to the conception and design of the study, collection and analysis of data, interpretation of findings, and drafting of the manuscript. Ankit Gupta contributed to the histopathological evaluation, interpretation of findings, and critical revision of the manuscript. Divya Jain contributed to study supervision,

interpretation of the results, critical revision of the manuscript, and final approval of the version to be published. All authors reviewed and approved the final manuscript.

Data Availability

The data generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare no conflict of interest.

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