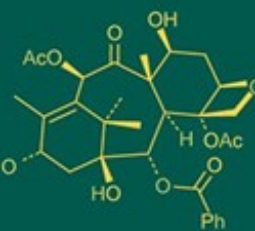
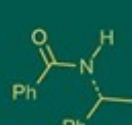


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
Indexing: Embase
IJABR 2026; 10(10): 250-254
www.biochemjournal.com
Received: 21-08-2026
Accepted: 28-09-2026
Published: 08-10-2026

The names and affiliations of all authors are provided below, following the references.

Critical analysis of the role of natural and synthetic dyes in veterinary science

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i10d.10358>

ABSTRACT

This article aims to critically analyze the role and application of natural and synthetic dyes within various branches of veterinary science. Through a comprehensive review of dye classifications and their specific uses, the study examines the utility of natural dyes like hematoxylin and carmine alongside synthetic options such as eosin, methylene blue, and Romanowsky-type stains. The results indicate that while natural dyes offer renewable sources, synthetic dyes are preferred for standardized diagnostic work due to their predictability and reproducibility. The analysis demonstrates that staining remains central to veterinary histopathology, microbiology, hematology, cytology, parasitology, ophthalmology, and oncology, evolving from simple contrast to molecularly targeted visualization. The authors conclude that natural and synthetic dyes are indispensable tools, and the future of the field lies in integrating these conventional methods with digital pathology, virtual staining, and sustainable green histopathology.

Keywords: Veterinary pathology, Histological stains, Natural dyes, Synthetic dyes, Diagnostic imaging

Introduction

Dyes are coloured substances capable of producing visible contrast when they bind to, react with, or are retained by biological structures. In veterinary science, dyes are fundamental to histopathology, cytology, hematology, microbiology, parasitology, ophthalmology, surgery, and experimental research. Routine and special stains transform otherwise low-contrast tissues or microorganisms into patterns that can be interpreted microscopically. The diagnostic value of a stain depends not simply on its color but on its chemical affinity, selectivity, reproducibility, fixation requirements and compatibility with the specimen. Natural dyes have been used since the early development of microscopy. Important examples include hematoxylin, carmine and orcein. Modern diagnostic laboratories, however, rely heavily on synthetic dyes because their composition can be standardized and their staining characteristics can be tailored. Examples include eosin, methylene blue, crystal violet, safranin, basic fuchsin, fluorescent dyes and components of Romanowsky stains. The distinction between natural and synthetic dyes is not always absolute; for example, carmine is a natural pigment complexed with a metal mordant, while hematoxylin requires oxidation to hematein before it functions effectively as a histological stain. The importance of dyes has increased rather than disappeared with modern pathology. H&E remains the basic morphological stain, while special stains and immunostains are selected when the routine pattern is insufficient to answer a diagnostic question. Recent veterinary studies continue to use histology and immunohistochemistry to characterize neoplasia and infectious disease, demonstrating that staining remains central even as molecular and digital techniques expand.

Classification of dyes used in veterinary science

Dyes can be classified according to source, chemical behavior, charge, staining mechanism and intended application. For practical veterinary work, classification by source is useful, while classification by staining behavior is often more useful diagnostically.

Natural dyes

- Hematoxylin – obtained from the heartwood of *Haematoxylum campechianum* and converted to hematein for effective staining.
- Carmine – derived from cochineal insects; carminic acid forms the basis of several carmine preparations.
- Orcein – traditionally derived from lichens and used particularly for elastic fibers.

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- Saffron, indigo and other plant-derived colorants – historically important and investigated as alternatives for histological staining.
- Plant-derived anthocyanins, curcuminoids, tannins and related pigments – investigated experimentally as sustainable staining agents (Cooksey, 2009; Hartika *et al.*, 2021; Alegbe *et al.*, 2024; Samanta *et al.*, 2023) [1, 2, 3, 4].

Synthetic dyes

- Acidic dyes: eosin and related xanthene dyes.
- Basic dyes: methylene blue, crystal violet, basic fuchsin and safranin.
- Romanowsky dyes: combinations of eosin with methylene-blue/azure derivatives, including Giemsa, Wright and Leishman stains.
- Lipid-soluble dyes: Sudan III, Sudan IV and Sudan Black B.
- Fluorescent dyes: fluorescein, acridine orange and rhodamine derivatives.
- Special histochemical reagents: Alcian blue, toluidine blue and components of PAS, trichrome and silver staining systems.

Natural dyes and their applications in veterinary science

Haematoxylin

Hematoxylin is one of the most important natural-origin dyes in diagnostic histology. It is obtained from logwood and must be oxidized to hematein before forming a coloured complex with a mordant. Aluminum hematoxylins are commonly used for routine nuclear staining, whereas iron hematoxylins are used in selected special applications. The resulting nuclear staining is blue to blue-purple and provides excellent definition of chromatin, nucleoli and nuclear membranes (Cooksey, 2010; Korzhevskii, 2007; Alturkistani *et al.*, 2016) [5, 6, 7].

In veterinary pathology, hematoxylin is most commonly used with eosin in H&E staining. H&E provides an overview of tissue architecture and is used for inflammatory, degenerative, necrotic, vascular and neoplastic lesions in domestic, laboratory and wild animals. The quality of nuclear staining is particularly important when assessing mitotic figures, nuclear atypia, apoptosis and inflammatory cell populations (Alturkistani *et al.*, 2016; Mescher, 2021) [7, 8].

Carmine

Carmine is derived from cochineal insects and contains carminic acid. Depending on the formulation and mordant, carmine can demonstrate nuclei, chromosomes, glycogen, or mucin and has historical importance in histology and parasitology. Its principal limitation is variability in the biological source and preparation, which can produce batch-to-batch differences. Modern use is therefore more specialized than routine H&E staining (Cooksey, 2009) [1, 9].

Orcein

Orcein is a natural dye traditionally obtained from lichens. It has been used to demonstrate elastic fibers and elastic lamellae. In veterinary pathology, elastic-fiber stains can assist in evaluating vascular lesions, pulmonary architecture, and connective-tissue changes. Although synthetic alternatives are now available, orcein remains relevant where an elastic-tissue stain is required.

Interest in plant-derived dyes has increased because many natural pigments are renewable and may reduce dependence on hazardous synthetic chemicals. Investigated sources include anthocyanin-rich flowers, turmeric-derived curcuminoids, hibiscus pigments, madder, annatto and indigo. Studies indicate that staining performance depends strongly on pigment concentration, pH, solvent, mordant, temperature and staining time (Alegbe *et al.*, 2024; Samanta *et al.*, 2023) [3, 4].

At present, most plant-derived dyes should be regarded as experimental or supplementary rather than replacements for standardized diag-

nistic stains. Their veterinary potential is greatest in teaching laboratories, research histology and situations where a low-cost local dye source is being evaluated.

Synthetic dyes and their applications in veterinary science

Eosin

Eosin is an acidic xanthene dye and the principal counterstain paired with hematoxylin in H&E. It colors cytoplasm, collagen, muscle, and many extracellular proteins in shades of pink to red, while erythrocytes are strongly eosinophilic. The contrast between blue-purple nuclei and pink cytoplasmic/extracellular components makes H&E an effective overview stain for routine veterinary diagnostic pathology (Alturkistani *et al.*, 2016; Mescher, 2021) [7, 8].

Methylene blue

Methylene blue is a basic thiazine dye with affinity for acidic cellular components. It is used alone for rapid bacterial or cytological staining and forms the basis of Romanowsky-type mixtures. In veterinary practice and teaching, methylene-blue-containing stains are useful for evaluating cells in smears, identifying bacteria and assessing cellular morphology.

Crystal violet

Crystal violet is a basic dye and the primary stain in Gram staining. Following iodine treatment, Gram-positive bacteria retain the crystal-violet-iodine complex after decolorization and appear purple, whereas Gram-negative organisms are counterstained pink/red. Gram staining provides a rapid preliminary classification of bacteria in specimens from wounds, otitis, mastitis, urinary infections, respiratory disease and other veterinary conditions.

Safranin

Safranin is commonly used as the counterstain in Gram staining. It colors decolorized Gram-negative organisms pink to red, improving contrast with Gram-positive bacteria. Safranin O is also used in histochemistry of cartilage because it binds to sulfated glycosaminoglycans and therefore provides information about proteoglycan distribution. This makes it useful in veterinary orthopedic research, particularly studies of cartilage degeneration and osteoarthritis (Little *et al.*, 2020) [10].

Basic fuchsin and carbol fuchsin

Basic fuchsin and carbol fuchsin are magenta synthetic dyes used in several microbiological and histochemical procedures. Carbol fuchsin is particularly important in Ziehl-Neelsen and related acid-fast methods. These stains assist in demonstrating acid-fast organisms, especially *Mycobacterium* species, which is important in veterinary diseases such as bovine tuberculosis and avian mycobacteriosis.

Romanowsky-type stains: Giemsa, Wright and Leishman

Romanowsky-type stains combine acidic and basic dyes and depend on methylene-blue/azure derivatives together with eosin. The characteristic purple color produced by the Romanowsky effect provides excellent contrast among leukocyte nuclei, cytoplasm and granules (Horobin, 2011) [11].

Giemsa is particularly valuable for blood parasites and cytology. Wright and Leishman stains are widely used for peripheral blood films and differential leukocyte counts. In veterinary hematology, these stains assist in recognition of erythrocyte morphology, leukocyte populations, platelet abnormalities and haemoparasites such as *Babesia*, *Theileria*, *Anaplasma* and other blood-borne organisms. They are also useful for cytological evaluation of inflammatory and neoplastic lesions.

Sudan dyes

Sudan III, Sudan IV and Sudan Black B are lysochrome dyes that stain lipids. They are useful for demonstrating neutral fat and other lipid classes in appropriately prepared specimens. Because routine

Natural dyes have advantages such as renewable biological sources and potential environmental benefits, but their composition can vary with species, geographical origin, season, extraction method and storage. Synthetic dyes generally provide more predictable color, concentration, and staining behavior and are therefore preferred for standardized diagnostic work. However, some synthetic dyes and associated reagents may pose occupational, toxicological or environmental hazards. Modern laboratory practice therefore requires appropriate ventilation, personal protective equipment, chemical storage and waste dis-

posal (Hartika *et al.*, 2021; Samanta *et al.*, 2023; Yalçın *et al.*, 2024) [2, 4, 20].

Advantages of dyes in veterinary diagnosis

- They increase contrast and make microscopic structures readily recognizable.
- They allow rapid preliminary identification of bacteria and blood parasites.
- They support tissue classification and lesion characterization.
- They help differentiate cellular populations in blood and cytological smears.
- They permit demonstration of specific substances such as collagen, mucin, glycogen, lipids, and elastic fibers.
- Fluorescent dyes allow high-sensitivity visualization and multiplex laboratory research.
- Staining is generally inexpensive compared with many advanced diagnostic technologies.
- Dye-based methods can be standardized and incorporated into routine diagnostic workflows.

Limitations and sources of error

Staining is not an infallible process. Fixation, tissue processing, section thickness, reagent age, pH, temperature, staining time, and differentiation can all influence the final appearance. Overstaining may obscure cellular details, whereas understaining may produce false impressions of reduced cellularity or weak antigen expression. Some stains also have limited specificity; a positive reaction should therefore be interpreted in the context of morphology and clinical findings (Alturkistani *et al.*, 2016; Horobin, 2011) [7, 11]. Natural dyes may show batch variation, and experimental plant dyes may lack standardized protocols. Synthetic dyes may involve hazardous solvents, mordants, or waste products. Laboratories should maintain standard operating procedures, internal quality-control slides, reagent logs and appropriate chemical-safety practices.

Emerging trends and future prospects

The future of staining is likely to combine conventional dyes with molecular, digital and computational approaches. Immunohistochemistry and immunofluorescence already permit target-specific visualization. Digital pathology can quantify staining intensity and spatial patterns, although inter-laboratory staining variation remains a challenge for image analysis and artificial intelligence (Getaneh, 2024) [12]. Virtual staining is an emerging approach in which computational methods generate images resembling conventional stains from unstained or differently imaged specimens. Recent work suggests that virtual H&E and other stain-conversion approaches may reduce reagent use and shorten some workflows, although validation, standardization, and diagnostic equivalence remain essential before routine clinical replacement (Modern Pathology, 2024) [21]. Another important direction is green histopathology. Research into plant-based pigments and other natural staining agents aims to reduce dependence on hazardous chemicals while maintaining acceptable staining quality. Natural dyes may eventually find a role in teaching laboratories, resource-limited settings and selected diagnostic applications, provided that reproducibility and validation are established (Alegbe *et al.*, 2024; Samanta *et al.*, 2023; Modern Pathology, 2024) [3, 4, 21].

Safety and good laboratory practice

- Use laboratory coats, gloves and eye protection when handling dyes, solvents and mordants.
- Prepare volatile or hazardous reagents in appropriate ventilation or fume-control systems.
- Label all stain solutions with name, concentration, preparation date and hazard information.
- Dispose of dye-containing and solvent-containing waste according to institutional and local regulations.
- Avoid direct skin contact and inhalation of powders or aerosols.
- Use validated staining protocols and quality-control slides.
- Do not interpret an isolated staining reaction without considering morphology and clinical information.

Dye/Stain	Type/Source	Principal target	Major veterinary use	Typical result
Haematoxylin	Natural-origin	Nuclei/chromatin	Routine histopathology	Blue-purple nuclei
Carmine	Natural	Nuclei/mucin/glycogen (depending on method)	Special histology/parasitology	Red to carmine
Orcein	Natural	Elastic fibres	Connective tissue/vascular pathology	Brown-red to purple
Eosin	Synthetic acidic	Cytoplasm/proteins	H&E	Pink-red
Crystal violet	Synthetic basic	Bacterial cell structures	Gram staining	Purple in Gram-positive bacteria
Safranin	Synthetic basic	Bacterial cells	Gram counterstain	Pink-red
Giemsa	Romanowsky mixture	Blood cells/parasites	Haematology, haemoparasites, cytology	Multicolour/purple-blue
Carbol fuchsin	Synthetic	Acid-fast organisms	Mycobacterial diagnosis	Red/pink organisms
Sudan dyes	Synthetic lipid-soluble	Lipids	Lipid histochemistry	Red/black depending on dye
PAS	Histochemical reaction	Carbohydrates/glycoproteins	Fungi, basement membranes, glycogen	Magenta
Alcian blue	Synthetic	Acidic mucins/GAGs	Mucin and cartilage studies	Blue
Toluidine blue	Synthetic metachromatic	Mast-cell granules/cartilage	Histology/cytology	Blue with metachromasia
Fluorescein	Fluorescent	Corneal epithelial defects	Veterinary ophthalmology	Green fluorescence
Acridine orange	Fluorescent	Nucleic acids	Cell/microbial research	Fluorescent contrast

Conclusion

Natural and synthetic dyes remain indispensable in veterinary science. Natural-origin stains such as hematoxylin and carmine established the foundation of biological staining, while synthetic dyes have enabled highly reproducible routine and special staining systems. Their applications span veterinary histopathology, microbiology, hematology, cytology, parasitology, oncology, ophthalmology, surgery, and research. H&E continues to provide the essential morphological overview, while special stains and fluorescent or immunohistochemical labels answer increasingly specific diagnostic questions.

The continued development of sustainable natural dyes, standardized special stains, digital image analysis and virtual staining indicates that the field is evolving rather than becoming obsolete. The most useful approach is therefore not to replace one category of dye with another, but to select the safest, most reproducible and diagnostically appropriate staining method for the veterinary question being investigated.

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How to cite this article: Kumar PS, Shukla M, Ranjan R, Lakshmi. K S, Reddy MA, Mewada R, et al. Critical analysis of the role of natural and synthetic dyes in veterinary science. *International Journal of Advanced Biochemistry Research*. 2026;10(10):250-254. doi: <https://www.doi.org/10.33545/26174693.2026.v10i10d.10358>

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