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REVIEW ARTICLE

A Comprehensive Review of Fixatives Used in Diagnostic Parasitology: Morphological Preservation, Technical Performance, and Modern Applications

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Abstract

Background: The selection of an appropriate stool fixative is pivotal for preserving parasite morphology and ensuring diagnostic accuracy in intestinal parasitology. Each fixative exhibits distinct advantages and limitations with respect to biosafety, compatibility with staining techniques, and applicability to molecular diagnostics. This review provides a systematic comparative evaluation of commonly used fixatives and offers evidence-based guidance to support their optimal selection in contemporary diagnostic laboratories.
Methods: A comprehensive literature search was performed across PubMed, Scopus, Web of Science, and Google Scholar to identify studies investigating fecal fixatives used in the diagnosis of intestinal parasites.
Results: 10% formalin remains the gold standard for helminth preservation but is unsuitable for delicate trophozoites and molecular assays. PVA and Schaudinn's fixatives provide excellent protozoan morphology yet are limited by mercury-related safety concerns. Mercury-free alternatives such as SAF, modified PVA, and single-vial systems offer safer and more versatile options, although with certain trade-offs in staining quality and consistency.
Conclusion: No single fixative meets all diagnostic needs. Choice should consider parasite type, laboratory setting, downstream applications, and safety regulations.

1. Introduction

Accurate laboratory diagnosis of intestinal parasitic infections remains a cornerstone of clinical parasitology and public health, particularly in low– and middle-income countries where these infections continue to impose a substantial disease burden [1-5]. Despite significant advances in molecular and immunological diagnostics, conventional stool microscopy remains the primary diagnostic approach in many routine laboratories worldwide [6].Within this context, the selection of an appropriate stool fixative is critical, as it directly influences parasite morphology preservation, diagnostic sensitivity, and the feasibility of applying complementary techniques such as concentration methods, permanent staining, and immunoassays.

Stool fixatives have been developed to address diverse diagnostic objectives, including the preservation of helminth eggs and larvae, protozoan cysts and trophozoites, long-term specimen storage, and safe sample transport. However, no single fixative is universally optimal for all diagnostic purposes. Each for-

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mulation exhibits distinct advantages and limitations related to parasite type, compatibility with staining procedures, suitability for concentration techniques, and interference with advanced diagnostic modalities, particularly nucleic acid–based assays. In addition, growing concerns regarding laboratory safety, environmental toxicity, regulatory restrictions, and cost-effectiveness have increasingly influenced fixative selection in modern diagnostic settings [7].

Historically, fixatives such as 10% formalin, polyvinyl alcohol (PVA), and Schaudinn's fixative have been regarded as reference standards due to their reliable preservation of specific parasitic stages [8]. Nevertheless, their limitations—including poor preservation of delicate protozoan trophozoites, incompatibility with molecular diagnostics, and the use of toxic mercury-containing compounds—have prompted the development of alternative mercury-free formulations. These include sodium acetate–acetic acid–formalin (SAF), modified PVA fixatives, merthiolate–iodine–formaldehyde (MIF), and more recently, one-vial commercial systems designed to integrate fixation, concentration, and permanent smear preparation within a single platform.

The present review provides a comprehensive and comparative evaluation of the most commonly used stool fixatives in diagnostic parasitology, including 10% formalin, MIF, low-viscosity PVA, SAF, Schaudinn's fixative, modified PVA formulations, and one-vial fixative systems. The advantages, limitations, and diagnostic applicability of each fixative are critically discussed with respect to morphological preservation, compatibility with staining and concentration techniques, safety considerations, and suitability for contemporary diagnostic workflows. This review aims to offer practical, evidence-based guidance to assist clinical laboratories and researchers in selecting the most appropriate fixative according to specific diagnostic requirements and operational constraints [9,10].

2. Methods

This systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews (PRISMA) guidelines.

2.1. Search Strategy

A systematic literature search was performed to identify relevant studies evaluating stool fixatives used in the diagnosis of intestinal parasitic infections. The following electronic databases were searched: PubMed/MEDLINE, Scopus, Web of Science, and Google Scholar. The search covered all publications from database inception to the final search date.

A combination of controlled vocabulary (MeSH terms) and free-text keywords was used. Search terms included: *stool fixative*, *fecal preservative*, *intestinal parasites*, *parasitological diagnosis*, *formalin*, *polyvinyl alcohol (PVA)*, *SAF*, *MIF*, *Schaudinn's fixative*, *modified PVA*, *one-vial fixatives*, *permanent staining*, *concentration techniques*, and *diagnostic microscopy*. Boolean operators ("AND", "OR") were applied to refine the search strategy. In addition, the reference lists of eligible articles and relevant review papers were manually screened to identify further pertinent studies.

2.2. Eligibility Criteria

Studies were included if they met the following criteria:

1. Original research articles, comparative studies, validation studies, or authoritative guidelines addressing stool fixatives used in human intestinal parasitology;
2. Evaluated one or more fixatives with respect to parasite morphology preservation, staining quality, concentration methods, diagnostic performance, or laboratory applicability;
3. Published in peer-reviewed journals;
4. Available in the English language.

2.3. Exclusion Criteria Were

1. Studies focusing exclusively on non-intestinal parasites or non-fecal specimens;
2. Conference abstracts, editorials, letters, and non–peer-reviewed sources lacking sufficient methodological detail;
3. Articles addressing fixatives not applicable to routine stool examination.

2.4. Study Selection

All retrieved records were imported into a reference management software, and duplicate entries were removed. Titles and abstracts were independently screened for relevance, followed by full-text assessment of potentially eligible articles. Discrepancies during the selection process were resolved through discussion and consensus. The study selection process is summarized using a PRISMA flow diagram.

2.5. Data Extraction

Data were systematically extracted from all included studies by the authors using a standardized data extraction form. Extracted variables included:

1. type and composition of fixative;
2. target parasite stages preserved;
3. compatibility with concentration techniques and staining methods;

4. suitability for immunological and molecular diagnostics;
5. safety, environmental, and operational considerations;
6. reported advantages and limitations.

2.6. Quality Assessment

Methodological quality and risk of bias were assessed based on study design and reporting clarity. Due to heterogeneity in study types and outcome measures, formal quantitative risk-of-bias tools were not uniformly applicable. Nevertheless, priority was given to well-designed comparative studies, standardized laboratory evaluations, and widely cited references from reputable journals. Studies with insufficient methodological transparency were interpreted cautiously.

2.7. Data Synthesis

Given the substantial heterogeneity across study designs, diagnostic techniques, and evaluated outcomes, a meta-analysis was not feasible. Therefore, results were synthesized using a qualitative narrative approach. Fixatives were categorized based on chemical composition and diagnostic application, and their performance was compared across key domains relevant to routine and advanced parasitological diagnostics.

2.8. Results

The systematic review included studies evaluating a range of stool fixatives commonly used in diagnostic parasitology. Overall, the evidence demonstrates that no single fixative is universally optimal for all diagnostic purposes; each exhibits specific advantages and limitations depending on the parasite type, the target life stage, compatibility with staining and concentration methods, suitability for molecular or immunological assays, and practical considerations such as preparation complexity, safety, and storage stability. The findings are therefore presented by fixative type, summarizing their chemical properties, diagnostic performance, and operational utility in both routine laboratories and field settings.

2.9. 10% Formalin

10% formalin is easily prepared without complex equipment, making it widely accessible. It offers excellent stability for long-term storage and effectively preserves the morphology of robust parasites like helminth eggs and protozoan cysts for reliable microscopic identification (Fig. 1). It is fully compatible with common concentration methods (e.g., formalin-ethyl acetate) and important staining techniques (e.g., acid-fast, chromotrope), enhancing diagnostic sensitivity and range. Furthermore, formalin-fixed specimens are suitable for immunoassays and UV fluorescence microscopy, supporting diverse diagnostic approaches [10-12]. 10% formalin is unsuitable for preserving delicate protozoan trophozoites, limiting diagnosis of infections like Entamoeba histolytica. It can also interfere with trichrome staining, reducing the quality of permanent stained smears. A major drawback is its negative impact on molecular assays, as it cross-links nucleic acids, making samples unsuitable for PCR and other DNA-based methods [11,12]. Despite these limitations, it remains a widely used fixative, but its use must be chosen carefully based on diagnostic needs, while it is considered the gold standard for preserving helminth eggs and larvae.

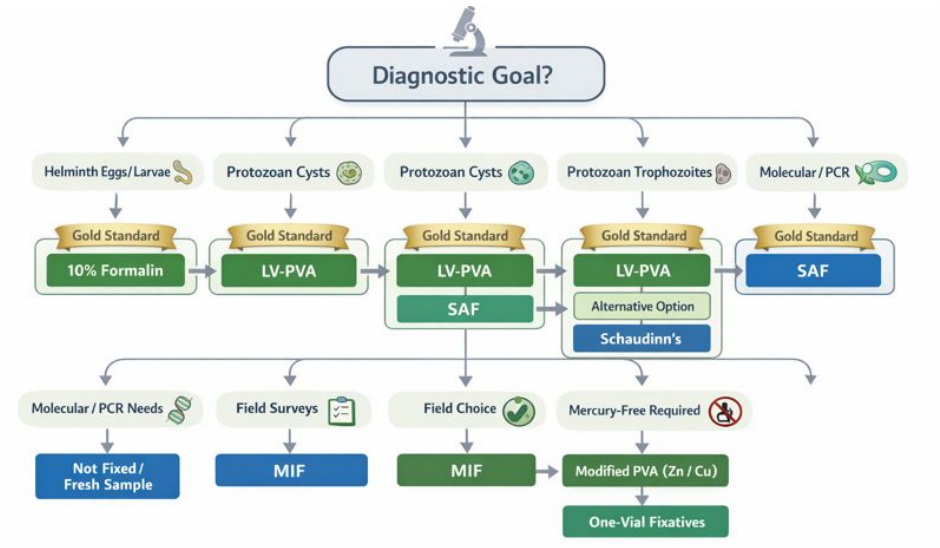

Fig. 1. Decision-making algorithm for the selection of fecal fixatives based on diagnostic objectives and environmental constraints.

3. Merthiolate–Iodine–Formaldehyde (MIF)

Merthiolate–Iodine–Formaldehyde (MIF) is a unique fixative valued in field and resource-limited settings, combining fixation and preliminary staining in one reagent due to its composition of a disinfectant, a stain, and a preservative, which distinguishes it and supports its widespread use in routine parasitology [13]. MIF is advantageous due to its simple preparation requiring only basic resources and its long chemical stability, ensuring cost-effectiveness and consistent availability. It is exceptionally useful in field surveys, as its combined fixative-and-stain function allows immediate specimen stabilization and preliminary visualization without additional reagents, aided by iodine for basic contrast. Furthermore, MIF remains compatible with common concentration methods like sedimentation, enhancing diagnostic sensitivity for detecting helminths and protozoan cysts even with low parasite burdens (Fig. 1). This makes it practical for both immediate field use and subsequent laboratory examination. A major limitation of MIF is its iodine-induced distortion of protozoan morphology, complicating species differentiation and making it unsuitable for high-fidelity morphological identification (Table 1). MIF-preserved samples are incompatible with essential techniques like trichrome staining and UV fluorescence microscopy due to poor coloration and interference. Furthermore, MIF is ineffective at preserving fragile protozoan trophozoites, limiting its diagnostic use for infections reliant on trophozoite morphology. Despite these limitations, MIF remains a practical and widely used fixative due to its ease of preparation, stability, and suitability for field work. Consequently, its limitations must be considered in routine clinical diagnostics, and alternative fixatives are preferred for detailed protozoan identification or advanced microscopy [13-16].

4. Low-Viscosity Polyvinyl Alcohol (LV-PVA)

Low-Viscosity Polyvinyl Alcohol (LV-PVA) is a benchmark fixative in parasitology, renowned for its excellent preservation of protozoan morphology and its ability to facilitate high-quality permanent stained slides, particularly for delicate trophozoites and cysts, maintaining its status despite newer mercury-free alternatives [10,17].

LV-PVA excels at preserving delicate protozoan trophozoites and cysts with minimal distortion, which is crucial for accurate microscopic identification (Fig. 1). It is highly compatible with permanent staining methods like trichrome, producing high-quality slides with sharp nuclear detail and consistent coloration, establishing it as the old gold standard for protozoan diagnosis. Additionally, LV-PVA provides exceptional long-term stability, allowing specimens to be stored for months without significant deterioration, making it reliable for reference labs, teaching, and quality assurance.LV-PVA is ineffective for preserving helminth eggs, larvae, coccidian oocysts, and microsporidian spores, often distorting their structural details. Its formulation contains toxic mercuric chloride, posing significant health, environmental, and costly disposal challenges, and requires complex, precise preparation. Furthermore, it is incompatible with modern diagnostic methods like immunoassays, concentration techniques, and key stains such as acid-fast and chromotrope, severely limiting its diagnostic utility but it still retains its status as a gold standard for protozoan morphology [1,17,18].

5. Sodium Acetate–Acetic Acid–Formalin (SAF)

Sodium Acetate–Acetic Acid–Formalin (SAF) is a mercury-free fixative developed for safety and practicality. Its key advantages are its versatility for both concentration techniques and permanent smears, a long shelf life with easy preparation, and compatibility with a wide range of staining methods including acid-fast, safranin, chromotrope, and immunoassays, making it valuable for routine diagnostics. The limitations of SAF include the frequent requirement for adhesives like albumin-glycerin to prepare permanent smears, complicating slide preparation, and its permanent stains are of slightly lower quality compared to PVA-based fixatives, potentially affecting detailed morphological observation (Table 1). Therefore, while it is a safe, versatile, and practical mercury-free fixative suitable for routine diagnostics, it does not fully replace specialized fixatives for all advanced applications [1,19,20].

Schaudinn's fixative is a historical diagnostic standard for protozoa, valued for its excellent preservation of fine morphological details in both trophozoites and cysts, and its ability to produce high-quality permanent stained smears for long-term reference and detailed analysis (Fig. 1).

6. Schaudinn's Fixative

Schaudinn's fixative contains toxic mercuric chloride, posing serious health and environmental risks, and is ineffective for preserving helminth eggs, larvae, coccidia, and microsporidia. It also has weak adhesion for mucoid samples and is unsuitable for concentration procedures. Therefore, while excellent for protozoan morphology and high-quality permanent smears, its toxicity and limited scope restrict its broader use, leading modern labs to prefer safer, more versatile alternatives [21,22].

Table 1
Comparative characteristics of commonly used stool fixatives in parasitological diagnosis.

Fixative	Main Advantages	Major Limitations
10% Formalin	• All-purpose fixative	Not suitable for some permanent smears stained with
	• Easy to prepare	• Trichrome
	• Long shelf life	•Inadequate preservation of morphology of protozoan trophozoites
	• Good preservation of morphology of helminth eggs, larvae, protozoan cysts, and coccidia	• Can interfere with PCR, especially after extended fixation time
	• Suitable for concentration procedures and UV fluorescence microscopy	
	• Suitable for acid-fast, safranin, and chromotrope stains	
	• Compatible with immunoassay kits and UV fluorescence microscopy	
MIF (merthiolate-iodine-formaldehyde)	• Components both fix and stain organisms	• Not suitable for some permanent smears stained with trichrome
	• Easy to prepare	• Inadequate preservation of protozoan trophozoites
	• Long shelf life	• Iodine interferes with other stains and fluorescence
	• Useful for field surveys	Iodine may cause distortion of protozoa
	• Suitable for concentration procedures	
LV-PVA (low polyvinyl-alcohol)	• Good preservation of morphology of protozoan trophozoites and cysts	• Inadequate preservation of morphology of helminth eggs and larvae, coccidia, and microsporidia
	• Easy preparation of permanent smears stained with such as trichrome (solution both preserves organisms and makes them adhere to slides)	• Contains mercuric chloride
	• Preserved samples remain stable for several months	• Difficult and expensive to dispose of
		• Difficult to prepare in the laboratory
		• Not suitable for concentration procedures
		• Cannot be used
		• Not suitable for acid-fast, safranin and chromotrope stains

SAF (sodium acetate-acetic acid-formalin)	• Suitable for both concentration procedures and preparation of permanent stained smears	• Requires additive (e.g., albumin-glycerin) for adhesion of specimens to slides
	• Easy to prepare	• Permanent stains not as good as with PVA or Schaudinn's fixative
	• Long shelf life	
	• Suitable for acid-fast, safranin, and chromotrope stains	
	• Compatible with s immunoassay kits	
Schaudinn's Fixative	• Good preservation of of morphology of protozoan trophozoites and cysts	• Less suitable for concentration procedures
	• Easy preparation of permanent stained smears	• Contains mercuric mercuric chloride
		• Inadequate preservation of larvae, coccidia, and microsporidia larvae, coccidia, and microsporidia
		• Poor adhesion of liquid or mucoid specimens to slides
Modified PVAc opper or zinc	• Permanent smears can be made stained with trichrome	• Staining not consistent
	Zinc is preferred over copper	• Organism morphology may be poor
	No mercuric chloride	• Copper-morphology of cysts and trophozoites is poor
		• Zinc-better morphology but not comparable to LV-PVA
One-Vial Fixatives (such as Ecofix, Parasafe, Unifix, Proto-fix, STF, and others that may be available	• Concentrate and permanent smear can be made made out of one vial	• Certain one-vial fixatives must use certain stains
	Immunoassays can be done on most	• Color difference of stain
	No mercuric chloride	• Staining not always consistent
		• Sometimes more expensive than formalin and LV-PVA

7. Modified PVA (Copper- or Zinc-Based)

Modified PVA fixatives are mercury-free alternatives developed to comply with environmental and regulatory restrictions. They are primarily used for preparing trichrome-stained permanent smears, with zinc-based formulas offering superior morphological preservation over copper-based ones for clearer visualization of protozoa, all while ensuring greater safety and environmental friendliness compared to traditional mercury-containing PVA. The key limitations of modified PVA fixatives are staining inconsistencies and morphological preservation inferior to LV-PVA, with copper-based

formulas performing most poorly (Table 1). As a safe, mercury-free alternative, they are suitable for routine diagnostics under regulatory restrictions, with zinc-based variants preferred for better clarity, though they do not match the standard smear quality of traditional PVA [1].

8. One-Vial Fixatives (Ecofix, Parasafe, Unifix, Proto-fix, STF)

One-vial fixatives are versatile, mercury-free systems that allow both concentration and permanent smear preparation from a single vial, streamlining workflow and reducing errors. They are compatible with most immunoassays, enhancing their diagnostic utility while improving laboratory safety and regulatory compliance.

One-vial fixatives often require specific staining protocols that may not align with all traditional methods, can lead to inconsistent staining outcomes, and are generally more costly, potentially limiting adoption in resource-constrained labs. Despite these drawbacks, they provide a modern, mercury-free solution for preparing both concentrated specimens and permanent smears from a single vial, streamline workflow, and maintain compatibility with most immunoassays, making them a safe and efficient choice for diagnostic laboratories prioritizing safety and modern workflow efficiency [1].

9. Discussion

The selection of an appropriate fixative is critical in stool parasitology as it directly impacts morphological preservation, diagnostic accuracy, and compatibility with complementary techniques such as permanent staining, immunoassays, and molecular methods. As illustrated in Table 1, no single fixative meets all diagnostic requirements; each exhibits inherent advantages and limitations dictated by its chemical composition and intended purpose.

10% Formalin remains widely used as a general-purpose fixative due to its long shelf life and effective preservation of helminth eggs, larvae, protozoan cysts, and coccidia. Its mechanism, involving cross-linking of proteins, stabilizes overall cellular architecture but often fails to maintain delicate protozoan trophozoites, leading to cytoplasmic shrinkage and nuclear detail loss, consistent with previous reports [10-12]. Furthermore, prolonged formalin fixation can interfere with nucleic acid extraction and downstream PCR-based diagnostics, limiting its utility for molecular applications.

In contrast, Merthiolate–Iodine–Formaldehyde (MIF) is particularly suitable for field surveys and rapid screening because it combines fixation and initial staining within a single solution, facilitating early detection of eggs and cysts, even in low-burden samples [13]. However, iodine content may induce osmotic distortion and denaturation of protozoan structures, rendering MIF unsuitable for permanent trichrome-stained smears or fluorescence microscopy. These limitations confine its use primarily to epidemiological surveys rather than high-resolution laboratory analyses.

According to the National Committee for Clinical Laboratory Standards, Low Viscosity Polyvinyl Alcohol (LV-PVA) and Schaudinn's fixative are considered gold standards for permanent protozoan smears due to superior preservation of nuclear and cytoplasmic details [21]. The effectiveness arises from adhesive properties that stabilize delicate protozoan structures and maintain their morphology on slides. However, both fixatives exhibit poor preservation of helminth eggs and larvae and are incompatible with concentration procedures, highlighting their niche applications. Additionally, the inclusion of mercuric chloride raises environmental and safety concerns, as disposal is complex and costly.

Mercury-free modified PVA formulations using copper or zinc attempt to address safety concerns. While eliminating toxic mercury, these formulations often compromise staining consistency and fine morphological preservation. Copper-based formulations particularly reduce the integrity of cyst and trophozoite morphology, whereas zinc provides better preservation but still falls short of LV-PVA quality [23].

Sodium Acetate–Acetic Acid–Formalin (SAF) presents a versatile, mercury-free alternative compatible with both concentration methods and permanent stains. Its chemical composition supports acid-fast, safranin, and chromotrope staining, as well as immunoassay compatibility. Nonetheless, permanent staining quality is inferior to PVA or Schaudinn, and adhesive additives like albumin-glycerin are required to maintain slide adherence, making it a practical but not ideal fixative for detailed morphologic studies [10,17].

Finally, one-vial fixatives represent modern solutions aimed at simplifying workflow, reducing handling errors, and maintaining immunoassay compatibility. By integrating concentration and permanent smear preparation in a single vial, these fixatives improve laboratory efficiency and standardization. Despite these advantages, reliance on proprietary stains, occasional inconsistencies in staining quality, and limited preservation of delicate protozoan trophozoites indicate that one-vial fixatives are not yet complete replacements for classical methods.

Overall, the literature supports a purpose-driven selection of fixatives based on target parasite type, diagnostic goals, laboratory resources, and downstream applications. In many cases, a combination of fixatives or targeted use of different fixatives for specific workflow steps (e.g., formalin for concentration, PVA

for permanent protozoan smears) remains the most effective strategy to maximize diagnostic sensitivity and specificity.

10. Conclusion

No single fixative can meet all diagnostic needs in intestinal parasitology. While traditional fixatives like 10% formalin and LV-PVA remain standards for specific applications, newer alternatives such as SAF, modified PVA, and one-vial systems provide safer and more versatile options. Optimal fixative selection depends on parasite type, target life stage, diagnostic method, and operational context. Future research should focus on developing integrated fixatives that combine morphological preservation, molecular compatibility, safety, and environmental sustainability, facilitating both routine diagnostics and advanced research applications.

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