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# Different fixatives give variable scenarios in terms of epitopes detection of CD68, CD24, calretinin, and TMEM119

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**Abstract**

Our study aimed to assess five commonly used fixatives-NBF, Davidson, Ethanol, Carnoy, and Bouin-in conserving epitopes of (CD68, CD24, TMEM119, and Calretinin). Twenty-five rats were housed at the College of Veterinary Medicine, University of Baghdad; five rats of each fixative were included with four organs representing our target IHC-specific marker of liver, intestine, brain, and testes. Using a scoring system based on the number of positively stained cells across 25 fields, antigen conservation is highly fixative- and marker-dependent. For CD68, Ethanol and Carnoy displayed the highest staining intensities, suggesting they are superior for maintaining key cellular markers. In contrast, NBF produced a balanced yet unspectacular spread of scores, while Davidson showed moderate effectiveness and Bouin tended to yield mid-range scores. Regarding CD24, most fixatives resulted low-to-moderate staining; however, Bouin distinctly produced a higher number of strongly stained cases, making it particularly effective for this marker. Regarding TMEM119, NBF and Davidson showed moderate, consistent staining, whereas Ethanol struggled, with many sections scoring low. Carnoy excelled by scoring few low-intensity scores and many high-intensity ones. Bouin's was a good mid-to-high alternative. NBF was least successful at preserving calretinin, with low-intensity staining. After Davidson balanced the distribution, Ethanol and Carnoy showed high intensity, substantially retained immunoreactivity, whereas Bouin's lagged. Immunohistochemical analysis should match the desired molecular detail or staining consistency. The study found that Bouin's fixative is best for CD24 detection, even if ethanol and Carnoy suit maintain antigens for other markers. NBF and Davidson excel at targeting markers based on study goals.

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## Introduction

Fixation is a vital step in immunohistochemistry (IHC), shaping the accuracy and consistency of staining outcomes. It preserves tissue architecture and prevents degradation, yet it can also modify antigenicity, influencing antibody binding efficiency (1). Crucial elements like the selection of fixative, its concentration, and the fixation time significantly influence immunoreactivity (2). Formalin fixation is frequently utilized for its capacity to preserve tissue integrity; nonetheless, it might result in antigen masking, necessitating antigen retrieval techniques to restore epitope accessibility. In the last twenty years, many research laboratories have attempted to substitute NBF with other fixatives that present reduced toxicity hazards (3). Nonetheless, these attempts have proven futile, chiefly because of the modifications detected in both cellular configuration and immunogenicity (4). Although each fixative has distinct advantages, it is crucial to acknowledge that their application is accompanied by several limits (5).

The extensive results further validated NBF as a suitable fixative for histology and immunohistochemistry (IHC)(1). Molecular investigations on fixed tissues encounter numerous obstacles, including the deterioration of DNA and RNA (6). Similarly, cellular organelle structures that are preserved can be changed as they have been in fixation for specific time, which can lead to differences in histochemical and immunohistochemical staining properties (7). To overcome these issues and improve IHC methods and achieve reliable diagnostic results, it's worthy to realize an adequate understanding of fixation effects (7). Lacking epitope access in NBF fixed paraffin-embedded (FFPE) tissue sections, coupled with less reachable amounts of target proteins, obstacles recognizing of cell surface determinants using immunohistochemistry (IHC) (8). Cluster of Differentiation (CD) Molecules are proteins that are located on the surface of cells, such as leukocytes and other types of cells. They serve an important role in controlling and performing of the immune system (7). CD68 is used to find macrophages in tissue sections, but it is still challenging to identify polarized macrophages in their natural environment. This challenge stems from the dynamic and varied characteristics of macrophage polarization, along with the constraints in identifying distinct functional states within the tissue milieu (9). CD24, a protein with several sugars on it, is an important marker for Paneth cells and intestinal crypt stem cells. Its expression is significantly elevated in inflammatory bowel disease (IBD), indicating a possible involvement in disease progression and immunological modulation (10). Calretinin, a calcium-binding protein, is widely expressed in both central and peripheral neural tissues. It has been identified as a useful marker in human testicular neoplasia, aiding in the characterization and diagnosis of certain testicular tumors (11). In addition, in the normal canine testes, calretinin expression is confined to Leydig and Sertoli cells, highlighting its specific role within testicular function and cell differentiation (Ref). Microglia, the resident myeloid cells of the central nervous system (CNS), become activated in various neurological diseases. They are ontogenetically and functionally distinct from monocyte-derived macrophages that infiltrate the CNS under pathological conditions. However, the lack of specific markers differentiating resident microglia from circulating blood-derived macrophages in human brain tissues complicates the precise assessment of microglial contributions to brain pathology. Through a comparative analysis of five extensive microglial transcriptome datasets, we identified TMEM119, an evolutionarily conserved protein, as the most promising marker for human microglia (12). Various fixatives interact differently with tissue components influencing antigen preservation, accessibility, and staining intensity. Some fixatives have little impact on detecting epitopes; however, others might cause masking or degradation, which make antigen retrieval mandatory to detect targeted epitopes. It is significant to know the variances of using different fixatives to improve both staining methods and immunohistochemistry to be read properly (5,13). This introduction focuses on the significance of fixative selection in ascertaining the consistency and specificity of epitope identification in histopathological investigation. Four IHC markers are CD68, CD24, Calretinin, and TMEM119 were chosen to evaluate the impacts of various fixatives in this assessment. Choosing the best fixative is challenging because it may be used in many ways and there are many factors that can affect their performance. In this investigation, we sought to determine which is the best and appropriate fixative based on various antigenic determinants.

## Materials and methods

### Ethical approval:

The University of Baghdad College of Veterinary Medicine Animal Care and Use Committee approved the experiments on animals under the license number (P.G/2084, October 28,2024).

### Experimental Design

Twenty-five adult male rats *Rattus norvegicus* were housed in the animal house of the College of Veterinary Medicine, University of Baghdad, and were of sexual maturity. we were used in this study; five rats for each fixative included four organs representing our target IHC-specific marker

Table 1: Fixatives and Their Associated Organ Markers

| Fixatives | Organs | IHC Markers | Target |
|---|---|---|---|
| NBF | Liver | CD68 | Kupffer cells |
| Davidson, Ethanol, | Intestine | CD24 | Paneth cells |
| Carnoy and Bouins | Brain | TMEM119 | Microglia |
| solution | Testes | Calretinin | Leydig cells |

Table 2: Details of IHC Markers

| Makers | Dilutions | Targeted cells | Catalogue No |
|---|---|---|---|
| CD68 | 1:200 | Kupffer cells | E-AB-64533 |
| CD24 | 1:100 | Paneth cells | E-AB-53309 |
| TMEM119 | 1:300 | Microglia | E-AB-13956 |
| Calretinin | 1:100 | Leydig cells | E-AB-19373 |

### Fixative Solutions Used

This study used five fixatives, prepared according to standard (14,15,16). The fixatives included neutral buffered formalin solution, Davidson's solution, ethanol solution, and Carnoy's and Bouin's solutions for 24 hrs. at 25ºC. Table 1 addresses the interaction between each fixative and its related organ and IHC marker.

**Tissue processing and sectioning**

The collected sections underwent a meticulous preparation process. Initially, they were fixed using a NBF, Davidson, Ethanol, Carnoy and Bouin's solution for 24 hours. Following fixation, the sections were dehydrated through a graded series of ethyl alcohol concentrations: 70% overnight, then 80% for two hours, 90% for another two hours, and finally 100% for two hours, with two changes. Subsequently, the tissues were cleared using xylene for 5 minutes in two changes. Next, they were embedded in hot paraffin wax at 55-58ºC for one and a half hour, with two changes. Once the paraffin block is formed, a rotary microtome was used to section the sections at a thickness of five µm (17,18,19).

**Immunohistochemistry**

Our study aimed to evaluate the impact of five widely used fixatives-NBF, Davidson, Ethanol, Carnoy, and Bouins solution on specific IHC markers commonly utilized in veterinary histopathology laboratories. We conducted an immunohistochemical analysis of the liver, intestine, brain, and testes of rat tissue sections, assessing the expression of CD68, CD24, TMEM119, Calretinin respectively following immersion in these fixatives as displayed in (Table 1).

Thin sections, measuring 5µm, were obtained from each tissue block and stained using commercially available antibodies. Specifically, CD68 Polyclonal Antibody (Elabscience Bionovation Inc, catalog #E-AB-64533) was used at a 1:200 dilution, while CD24 Polyclonal Antibody (Elabscience Bionovation Inc, catalog #E-AB-53309) was applied at a 1:100 dilution. Additionally, TME19 Polyclonal Antibody (Elabscience, catalog #E-AB-13956) was used at a 1:300 dilution, and CALB2 Polyclonal Antibody (Bionovation Inc, catalog #E-AB-19373) was applied at a 1:100 dilution. Staining was conducted on a Leica Bond immunostainer according to the manufacturer's instructions (Table-2). (20,21,22) To assess antibody immunoreactivity with rat antigens, sections from twenty-five rats were examined.

**IHC scoring criteria**

A scoring system ranging from 0 to 3 was established, with the corresponding scoring scale detailed in Table 3. The results for each marker were derived from an analysis of 25 microscopic fields. The average of each 25-counting field determines the average score of each marker with its associated fixative (7,23,24).

Table 3: Showing the Score System Used in This Study

| Scores | Positive Cells Were Detected |
|---|---|
| 0 | No |
| 1 | Positive cells < 20 cells |
| 2 | 20 > Positive cells < 30 cells |
| 3 | Positive cells > 30 cells |

**Statistical Analysis**

Descriptive analysis was performed using JMP® Pro 16.1 (25) to summarize and organize the collected data. Frequencies and proportions were calculated for each score associated with each fixative.

**Results**

**CD68 Immunohistochemistry Records**

The IHC findings demonstrated statistically significant differences in scoring criteria both within individual scores and across different scores among all fixatives. This variation was observed in relation to positive staining immunoreactivity and the number of positively stained cells, which reflected immune reactivity. Figures 1 and 2 display that Ethanol and Carnoy show robust performance at the highest score (16 and 12, respectively), suggesting that they may excel in preserving specific cellular markers or structures critical for analysis. NBF demonstrates a balanced progression across scores, meaning it might provide consistent and reliable tissue preservation without extreme variations. Davidson has a peak at score 3 but remains even across other levels, indicating moderate effectiveness across different scoring criteria. Bouin's fixative has the most concentrated scores in the mid-range (Score 1 and 2), dropping at Score 3, which suggests it may work well in certain conditions but could lose effectiveness in more stringent requirements. This analysis could help in choosing a fixative based on the required tissue characteristics or staining properties. For example, Ethanol might be preferable if the goal is preserving specific molecular details, while NBF could be a solid choice for overall consistency.

Table 4: CD68 Scoring results

| Scoring of Each Fixative | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| NBF | 2 | 5 | 7 | 11 |
| Davidson | 2 | 8 | 6 | 9 |
| Ethanol | 1 | 2 | 6 | 16 |
| Carnoy | 1 | 4 | 8 | 12 |
| Bouin's | 6 | 9 | 6 | 4 |

**CD24 Immunohistochemistry Records**

Figures 3 and 4 exhibit that many sections fixed with NBF showed little or moderate CD24 staining, as the high count at score 0 (10 sections) and score 1 (9 sections) demonstrates limited antigen detection. Only 1 section showed a strong (score 3) reaction, implying that while NBF is dependable, it might not be optimal for high-intensity CD24 preservation.

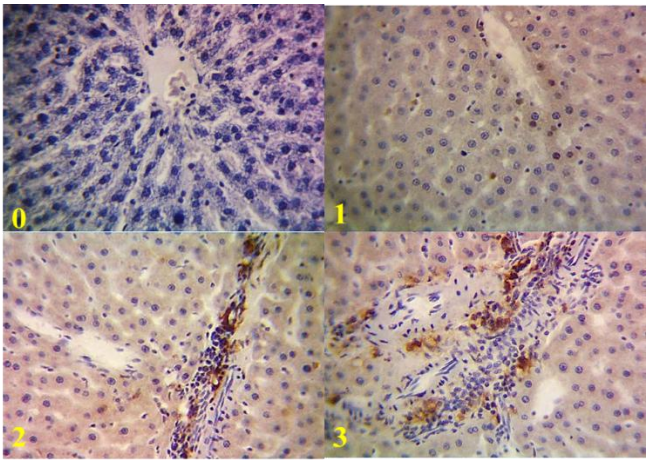


Figure 1: The scoring system's catalog: Immuno histochemical examination of positive reaction against CD68 marker in the hepatic tissue of rats The upper-left section represents a score of zero, while the upper-right indicates a score of one. The lower-left corresponds to score two criteria, and the lower-right panel denotes a score of three. 400X.

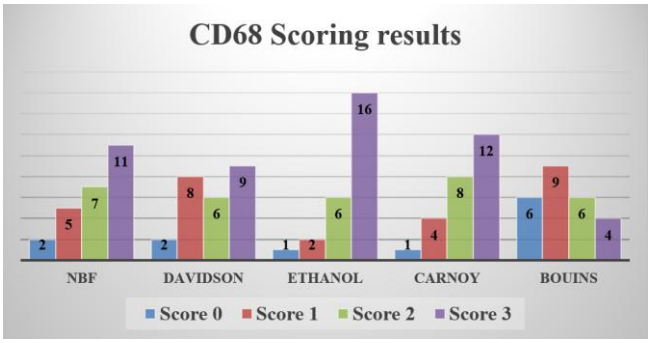


Figure 2: Visually represents the CD68 scoring results across different fixatives (NBF, Davidson, Ethanol, Carnoy, and Bouin's).

Regarding Davidson's, sections showed the highest score for score 0 (10 sections), and very few sections in the higher intensity ranges. This pattern suggests that Davidson may lead to a loss of antigenicity for CD24, making it less effective if strong positive staining is required. While with Ethanol, we again observed a strong leaning towards score 2 (10 section's) and only minimal representation in the lower scores. On the other hand, Carnoy shows an interesting trend. While 9 sections were at score 0, there was a peak at score 1 (12 sections), suggesting moderate levels of CD24 preservation are more frequently achieved. However, like the others, very few tissues reached a strong staining level (only 1 section at Score 3). Bouin's stand out in this analysis. Although it had a lower count in score 0 compared to the others, it dramatically increased at score 3 with 10 sections. This suggests that Bouin is particularly effective in preserving or revealing CD24 staining in a strong, clear manner. Overall, Bouin's emerges as the most effective based on this data it consistently produces more high-intensity (score 3) signals. The other fixatives (NBF, Davidson, Ethanol, and Carnoy) tend to cluster toward low or moderate scores, suggesting they might be less optimal if high antigen detectability is desired.

Table 5. CD24 Scoring results

| Scoring of Each Fixative | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| NBF | 10 | 9 | 5 | 1 |
| Davidson | 10 | 11 | 2 | 2 |
| Ethanol | 2 | 5 | 10 | 8 |
| Carnoy | 9 | 12 | 3 | 1 |
| Bouin's | 2 | 5 | 8 | 10 |

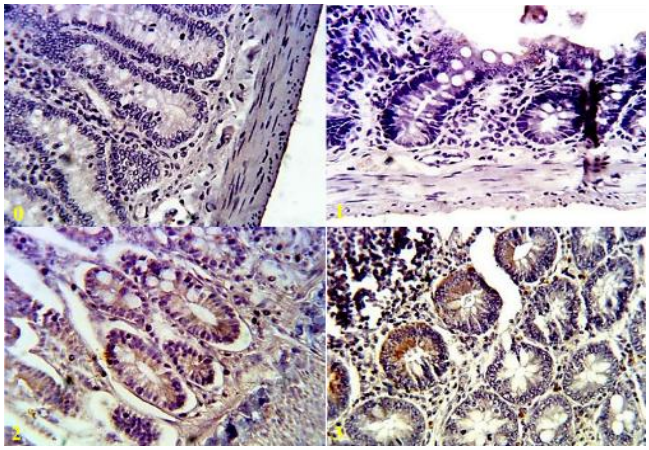


Figure 3: The scoring system's catalog: Immuno histochemical examination of positive reaction against CD24 marker in the small intestine of rats. The upper-left section represents a score of zero, while the upper-right indicates a score of one. The lower-left corresponds to score two criteria, and the lower-right panel denotes a score of three. 400X.

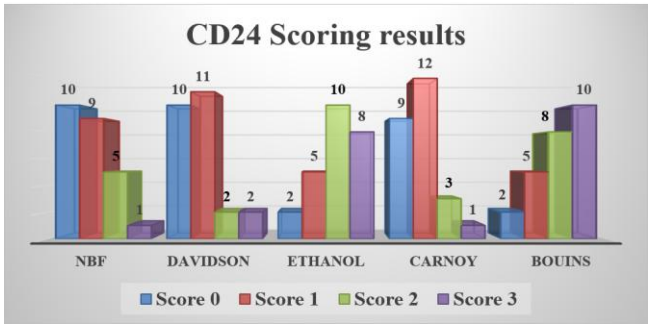


Figure 4: The fixative effectiveness regarding CD24 immunoreactivity.

**TMEM119 Immunohistochemistry Data**

Figures 5 and 6 show that NBF had a balanced spread across all scores, peaking at score 1 and score 2 (9 sections each), but showing a low count at score 3 (only 1 section). This suggests that NBF provides moderate preservation but is not optimal for achieving strong staining. Davidson follows a similar pattern, peaking at score 1 (10 sections) and score 2 (8 sections), while showing a slight increase at score 3 (2 sections). This indicates Davidson may allow stronger staining than NBF. Ethanol had a high concentration at score 0 (10 sections) but evenly distributed among scores 1-3. This pattern implies ethanol struggles to preserve antigenicity but can still allow some degree of staining. Carnoy excelled, it had an extremely low count at score 0 (1 section), peaks strongly at score 3 (11 sections), and Score 2 (9 sections). This suggests Carnoy is one of the most effective fixatives at preserving TMEM119. Bouin's also maintained a high score 2 count (10 sections) with a moderate score 3 (3 sections), making it another fixative that supports stronger staining. Carnoy appears to be the strongest performer, showing the highest retention of antigenicity for TMEM119. Bouin's also performed well, although slightly less effective in yielding high staining intensity compared to Carnoy. NBF, Davidson, and Ethanol exhibit weaker staining, clustering mostly in mid-range scores, with lower counts for the highest preservation. Carnoy might be the best choice, while Bouin's could be a strong alternative.

Table 6. TMEM119 Scoring results

| Scoring of Each Fixative | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| NBF | 6 | 9 | 9 | 1 |
| Davidson | 5 | 10 | 8 | 2 |
| Ethanol | 10 | 8 | 5 | 2 |
| Carnoy | 1 | 4 | 9 | 11 |
| Bouin's | 3 | 9 | 10 | 3 |

**Calretinin Immunohistochemistry Data**

Figures 7 and 8 in NBF, many tissues are scored at 0 (12 out of 25), and only 1 section reaches the highest score. NBF appears to be less effective in preserving calretinin antigenicity, resulting in weak or no staining. Davidson- the scores were more balanced, with the highest count at Score 1 (8 out of 25) and a moderate 4 sections at Score 3. It shows better performance than NBF but still produces only a modest number of strong staining results. While Ethanol, there were fewer tissues with score 0 (1), a standout is the highest number of tissues with score 3 (13 out of 25). Ethanol is highly effective at preserving calretinin, given that almost half of the sections show strong immunostaining. On the other hand, Carnoy, like Ethanol, Carnoy yields 13 sections at score 2 (out of 25), with small numbers for score 0. Carnoy is also highly effective, matching Ethanol in producing strong calretinin positivity. Regarding Bouin's, it exhibits a higher count at score 2 (9) and a moderate distribution among scores 1 and 2. Only 4 sections reach score 3. Bouin's appeared less effective compared to Ethanol and Carnoy; its distribution indicates that many tissues do not achieve strong calretinin staining.

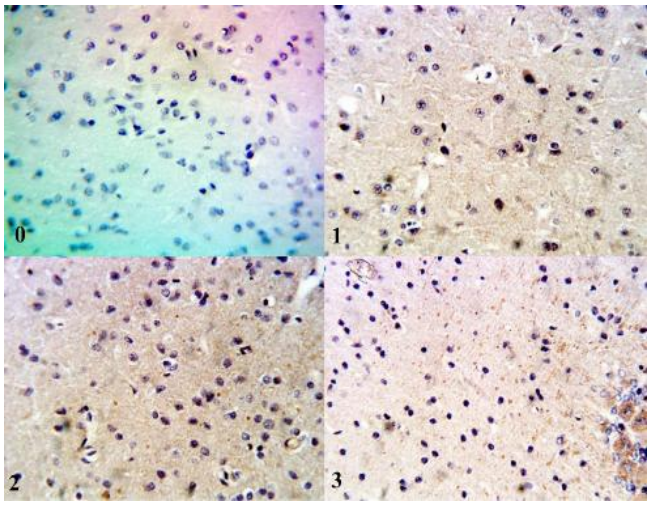


Figure 5: The scoring system's catalog: Immuno histochemical examination of positive reaction against TMEM119 in the Brain tissue of rats. The upper-left section represents a score of zero, while the upper-right indicates a score of one. The lower-left corresponds to score two criteria, and the lower-right panel denotes a score of three. 400X.

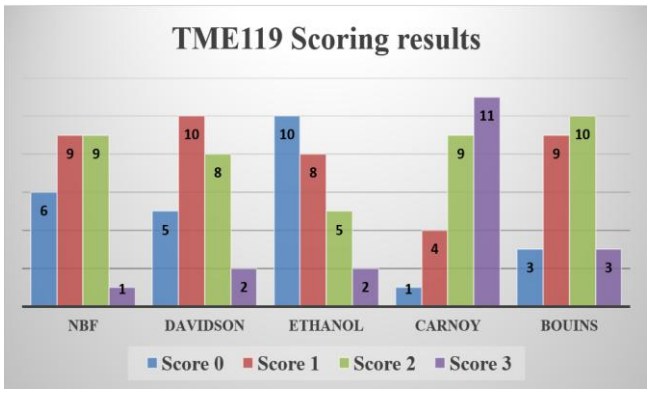


Figure 6: TMEM119 scoring results.

Table 7: Calretinin Scoring results

| Scoring of Each Fixative | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| NBF | 12 | 6 | 6 | 1 |
| Davidson | 4 | 8 | 9 | 4 |
| Ethanol | 1 | 5 | 6 | 13 |
| Carnoy | 3 | 4 | 13 | 5 |
| Bouin's | 6 | 6 | 9 | 4 |

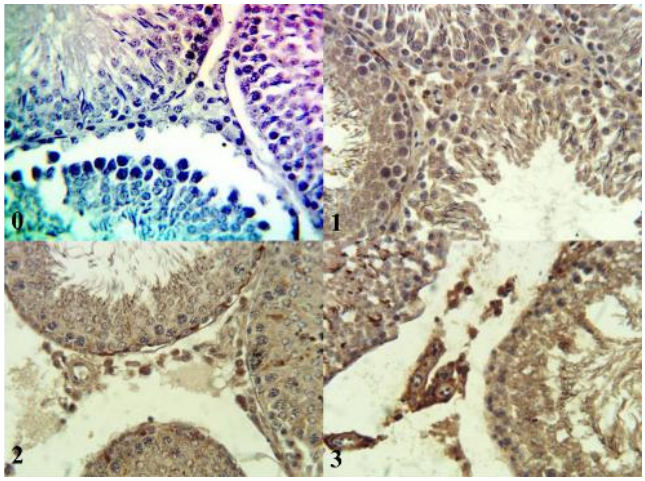


Figure 7: The scoring system's catalog: Immunohistochemical examination of positive reaction against Calretinin in the testis tissue of rats The upper-left section represents a score of zero, while the upper-right indicates a score of one. The lower-left corresponds to score two criteria, and the lower-right panel denotes a score of three. 400X.

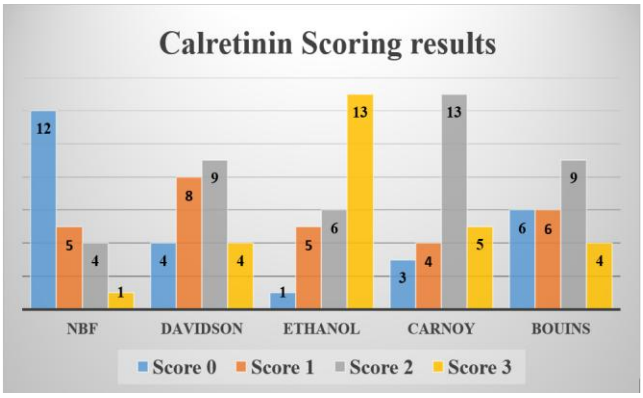


Figure 8: Analysis of the Calretinin Scoring Results.

**Discussion**

Fixation is a vital step in IHC, shaping the accuracy and consistency of staining outcomes. It preserves tissue architecture and prevents degradation, yet it can also modify antigenicity influencing antibody binding efficiency (1) Our analysis corresponds with results from several research about fixative efficacy. Buffered Ethanol Fixative and other ethanol-based fixatives are known for their ability to keep molecules intact, especially RNA and DNA. This makes them good for molecular applications (26). Carnoy's fixative, which has ethanol and acetic acid in it, is known for its powerful performance in maintaining cellular markers, especially in histological staining (27) these findings align with our data. On the other hand, NBF is extensively utilized for its effective preservation properties, maintaining uniform tissue integrity throughout various score levels. Davidson's fixative, although moderately effective, is frequently utilized for particular tissue types, such as ocular and testicular tissues, where structural integrity is paramount (27). Our results are concise for what have been mentioned by (27). Bouin's fixative, which exhibits elevated scores in the mid-range, is recognized for its capacity to improve staining contrast, although may diminish in efficacy under rigorous settings (7,27). The examination of CD24 staining across several fixatives corresponds with findings from multiple studies on fixation techniques. NBF is extensively utilized for its effective preservation properties; nevertheless, evidence indicates it may not be ideal for high-intensity antigen detection. Studies indicate that formalin-based fixatives can mask antigenic sites, requiring antigen retrieval techniques for effective staining (5). Davidson's fixative, commonly used for ocular and testicular specimens, has been reported to cause antigen loss in certain tissues. This aligns with our observation that Davidson's fixative leads to weak CD24 staining, making it less effective for strong positive staining (28, 29). Ethanol-based fixatives, including Carnoy's, are known for their ability to preserve nucleic acids but may not be ideal for protein antigen preservation. Our findings that Carnoy achieved moderate CD24 staining but lacked strong signals are consistent with reports that ethanol-based fixatives can lead to weaker antigen detectability. Bouin's fixative, on the other hand, has been recognized for its ability to enhance staining contrast and preserve antigenicity. These results show that Bouin's fixative produced the highest intensity CD24 staining (score 3) aligning with studies that highlight its effectiveness in maintaining strong antigen signals (30, 31). Overall, this analysis supports existing literature on fixative effectiveness. Bouin's fixative is the most effective for robust CD24 staining, whereas NBF, Davidson, Ethanol, and Carnoy provide weaker signals. These insights assist researchers in selecting the most suitable fixative according to antigen detectability criteria. Our evaluation of fixative efficacy in maintaining TMEM119 antigen staining corresponds with results from histological investigations (32). NBF exhibited a uniformly distributed over scores, reaching a zenith at score 1 and score 2, while exhibiting minimal robust staining. This indicates that although NBF offers reasonable preservation, it may not be ideal for attaining high detection of antigen intensity. Research demonstrated that formalin-based fixatives may obscure antigenic sites. necessitating retrieval techniques for efficient staining (32). Davidson's fixative exhibits a comparable trend, peaking at score 1 and score 2, with a marginal rise at score 3 (33). This indicates that Davidson may permit more intense staining than NBF, although still demonstrates constraints in antigen preservation. Davidson's fixative is commonly employed for tissue types such as ocular and testicular tissues, where structural integrity is crucial. Ethanol-based fixatives, such

as Carnoy's, exhibit notable tendencies. Ethanol exhibits difficulty in maintaining antigenicity, as evidenced by its elevated concentration at score 0, although permits a certain level of staining across scores 1-3. Carnoy, however, is distinguished by low counts of score 0, exhibiting a pronounced rise at score 2 and score 3, indicating it is among the most efficacious fixatives for retaining TMEM119 antigen staining. Studies highlight Carnoy's ability to maintain nucleic acid integrity while supporting antigen detectability. Bouin's fixative also performed well, maintaining a high score 2 count with moderate score 3 representation. Bouin's fixative is recognized for its ability to enhance staining contrast and preserve antigenicity, making it a strong alternative to Carnoy. Overall, Carnoy appears to be the strongest performer, showing the highest retention of antigenicity for TMEM119, while Bouin's serves as a reliable alternative. NBF, Davidson, and Ethanol exhibited weaker staining, clustering mostly in mid-range scores, with lower counts for the highest preservation. If achieving high-intensity antigen staining is the goal, Carnoy would be the preferred choice followed by Bouin's for enhanced staining contrast (32, 34, 35, 36). Our results regarding calretinin antigen staining across different fixatives affiliate with findings from histopathological studies on fixation effectiveness. NBF appears to be the least effective in preserving calretinin antigenicity, as indicated by the high number of tissues scored at 0 and only one section reaching the highest score. This is consistent with research showing that formalin-based fixatives can mask antigenic sites requiring retrieval techniques for effective staining (36). Davidson's fixative demonstrated a more balanced distribution, with the highest count at score 1 and a moderate number of sections at score 3. While Davidson performed better than NBF, it produced only a modest number of strong staining results. Research indicated that Davidson's fixative is frequently employed for particular tissue types, including ocular and testicular specimens, where the preservation of structure is emphasized. Ethanol-based fixatives, such as Carnoy's, demonstrated significant efficacy in maintaining calretinin antigenicity. Ethanol exhibited a lower number of tissues at score 0 and the highest quantity at score 3, demonstrating its efficacy in preserving robust immunostaining. Carnoy's fixative exhibited comparable efficacy to Ethanol in eliciting robust calretinin positivity, as evidenced by 13 sections achieving a score of 3. Research underscores Carnoy's capacity to preserve nucleic acid integrity while enhancing antigen detectability. Bouin's fixative demonstrates a higher frequency at score 0 and a moderate distribution across scores 1 and 2, with only 4 sections attaining score 3. This indicates that Bouin's solution is less effective than Ethanol and Carnoy in preserving calretinin antigenicity (31). Bouin's fixative is acknowledged for improving staining contrast; however, its capacity to preserve robust antigen signals seems constrained in this context.

## Conclusion

Ethanol and Carnoy's fixatives exhibited strong antigen preservation for various markers, rendering them appropriate for high-fidelity immunostaining applications. Bouin's solution demonstrated notable advantages for the optimal staining of CD24, indicating its relevance in research centered on this specific antigen. In contrast, NBF and Davidson's fixative offered moderate and consistent preservation of antigens. These fixatives may be advantageous in situations where extreme staining intensity is not critical, but reproducibility and overall staining quality are emphasized.

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## Conflict of interest

The authors disclosed no conflicts of interest.

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# المثبتات المختلفة تُنتج سيناريوهات متباينة في الكشف عن المحددات المستضدية لـ CD68 وCD24 والكالريتينين وTMEM119

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**الخلاصة**

هدفت الدراسة إلى تقييم خمس مثبتات نسيجية شائعة الاستخدام وهي (الفورمالين المتعادل، المحايد، محلول ديفيدسون، الإيثانول، محلول كارنوي، ومحلول بوين) في الحفاظ على فعالية مؤشرات الكيمياء النسيجية المناعية (CD68، CD24، TMEM119، وCalretinin). شملت التجربة ٢٥ جرذًا بالغًا في بيت الحيوان التابع لكلية الطب البيطري بجامعة بغداد، بواقع خمسة جرذان لكل مثبت، وتم تحليل أربعة أعضاء (الكبد، الأمعاء، الدماغ، والخصى) لتمثيل المؤشرات المستضدية المستهدفة. اعتمد التقييم على معايير قياسية في عد الخلايا الموجبة للتلوين المناعي الكيميائي النسيجي في ٢٥ مقطعا مجهريًا لكل عينة. أظهرت النتائج أن حفظ المستضدات يعتمد على نوع المثبت والمحدد المســتضدي. تفوق الإيثانول ومحلول كارنوي في الحفاظ على CD68،

بينما قدم الفورمالين المتعادل توزيعًا متوازنًا، وسجل محلول ديفيدسون شدة متوسطة. بالنسبة لـ CD24، تميز بوين بتفاعل مناعي نسجي، مقارنة ببقية المثبتات التي أظهرت تلوينًا منخفضًا إلى متوسط. في مؤشر TMEM119، أظهر كل من الفورمالين المتعادل ومحلول ديفيدسون تفاعلا مناعيا متوسطا، بينما سجل الإيثانول درجات منخفضة، وتفوق محلول كارنوي بتفاعل مناعي كيميائي نسجي عالي الكثافة. أما محلول بوين فكان أداؤه متوسطًا إلى جيد. بالنسبة لـ Calretinin، كان الفورمالين الأقل فعالية، بينما قدم محلول ديفيدسون توزيعًا متوازنًا، وتفوق كل من الإيثانول ومحلول كارنوي بكثافة عالية، في حين تضاءل التفاعل المناعي النسيجي لمحلول بوين نسبيًا. تستنتج الدراسة أهمية اختيار المثبت وفقًا لهدف التحليل المناعي الكيميائي النسيجي حيث يوفر الإيثانول ومحلول كارنوي حفظًا قويًا لمعظم المؤشرات، بينما يُعد بوين خيارًا مثاليًا لـ CD24، وتناسب فعالية الفورمالين ومحلول ديفيدسون التطبيقات ذات المتطلبات المتوسطة.