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OPTIMIZING FIXATION DURATION FOR ENHANCED CLARITY IN *Pediculus humanus-capitis* WHOLE MOUNT PREPARATIONS USING 10% KOH AT 70°C

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ABSTRACT

This study evaluates the optimal fixation duration using 10% potassium hydroxide (KOH) at 70°C for whole-mount preparations of Pediculus humanus-capitis (head lice). Accurate head lice identification is crucial for effective public health management. The specimens were fixed for varying durations (20, 25, 30, 35, and 40 minutes) at 70°C, then processed through rinsing, acetic acid treatment, dehydration, and clearing before mounting for microscopic examination. The results showed that 25 minutes of fixation provided the most precise and detailed preparations. This duration effectively achieved tissue dehydration and clarification without significant damage. Fixation for 20 minutes was insufficient, resulting in opaque specimens due to incomplete KOH diffusion.

INTRODUCTION

Pediculus humanus-capitis, commonly known as head lice, is a cosmopolitan ectoparasite that inhabits and reproduces on the human scalp (Azizah et al., 2022). It directly feeds by extracting nutrients from the venules of the scalp (Bharti et al., 2017). The eggs of Pediculus humanus capitis are ovoid, with the final third of the egg attached to the hair shaft, forming nit sheaths of varying lengths that partially encircle the hair (Álvarez-Fernández et al., 2023). Head lice secrete substances that cause tissue irritation

on the scalp, leading to intense itching (Hayati, 2019). *Pediculus humanus capitis* can also cause symptoms such as fatigue, irritation, paranoia, anemia, and conjunctivitis. These symptoms result from repeated lice bites, with the continual inoculation of lice saliva, which contains vasodilators and anticoagulants, potentially triggering allergic reactions and itching. Infestations of *Pediculus humanus-capitis* can also lead to psychological impacts, including feelings of shame, low self-esteem, isolation, fear, and even frustration due to societal stigma associating head lice with poor hygiene, poverty and a lack of parental care. The psychological distress caused by these infestations can negatively affect an individual's well-being, performance, and academic achievement, particularly in infested students (Widniah et al., 2019; Massie et al., 2020).

Controlling and identifying head lice is crucial for public health, especially in epidemiology and infestation management. In the educational domain, the creation of wholemounts also plays a significant role. Mastering preparation techniques enhances teachers' abilities to create effective teaching media. Harlita et al. (2018) noted that many teachers rely on easily accessible online resources, such as images of animal and plant tissue or organ slides, due to a lack of skills in producing educational media. However, when teachers employ creativity and incorporate direct observation, it can significantly enhance student understanding and engagement. Noviyanti et al. (2023) propose that utilizing 3D learning media with realistic appearances is an effective strategy for improving material retention and comprehension. One particularly engaging and realistic medium is whole mounts, including those of Pediculus humanus capitis. Thus, mastering the creation of educational media is essential for teachers who aim to enrich the learning experience.

Mikroteknik, or preparing specimens from animals or plants, is fundamental in studying tissues in conditions that closely resemble their original state, with minimal post-mortem changes (Faluti et al., 2022). This technique allows for examining various tissue and cell components in well-differentiated conditions. One method within mikroteknik is wholemount preparation, which involves fixing specimens in a potassium hydroxide (KOH) solution to clear soft tissues, thereby facilitating the observation of morphological structures. KOH fixation is a process that uses a potassium hydroxide solution to prepare specimens for microscopic examination (Susetyarini et al., 2020; Hidayani et al., 2018). Recent studies on KOH fixation methods have produced mixed results concerning the

quality of wholemount preparations and optimal fixation times. While previous research has varied KOH concentrations and temperatures, it has largely yet to investigate the effects of varying fixation durations at a constant temperature (Azizah et al., 2022). While some studies have tested the effectiveness of different KOH concentrations in enhancing the quality of *Pediculus humanus-capitis* whole mounts, there needs to be more literature regarding how varying fixation durations at a constant temperature influence the outcome. Most research focuses on a single parameter, such as KOH concentration or temperature, without exploring how varying the fixation time at a consistent temperature affects wholemount quality. This underscores the need for more detailed research on the optimal fixation duration using 10% KOH at 70°C. Determining the optimal fixation time is crucial for improving standard laboratory techniques and achieving more accurate and reliable identification results.

The primary aim of this study is to evaluate the effect of varying fixation durations with 10% KOH at 70°C on the quality of *Pediculus humanus-capitis* whole mounts. The study also aims to provide practical recommendations for laboratories to determine more efficient and effective fixation procedures for head lice observations. This research is expected to contribute significantly to ectoparasite identification techniques, which are methods for identifying parasites that live on the outside of their host's body, and improve head lice infestation control methods in clinical and laboratory settings by exploring optimal fixation durations.

MATERIALS AND METHODS

This experimental study, conducted in the State University of Manado Biology Laboratory on 6 May 2024, utilized advanced technology and modern techniques. The procedure began with collecting *Pediculus humanus-capitis* and placing them into a glass vial. The specimens were then fixed by immersing them in a 10% KOH solution at 70°C for varying durations of 20, 25, 30, 35, and 40 minutes. After fixation, the specimens were transferred to a watch glass and rinsed with distilled water for 10 minutes. They were treated with 10% acetic acid for 30 minutes, followed by another 10-minute rinse with distilled water. The specimens were then dehydrated using alcohol solutions of 50%, 70%, and 96% for 10 minutes each. Next, they were immersed in

clove oil for 30 minutes and mounted between a slide and cover slip to remove air bubbles. The specimens were treated with pure xylene for 30 minutes and then transferred to a slide for microscopic observation, with additional xylene added as needed. Once the overall shape of the specimens was observed, a mounting medium was applied, and the slide was covered with a cover slip. The specimens were photographed at magnifications of 4x and 10x, and their evaluation was carried out using a Google Form survey with responses from 26 faculty members and educators.

RESULTS AND DISCUSSION

This study, with practical implications for whole-mount preparation techniques, aims to assess the impact of fixation using a 10% KOH solution at various time durations on the clarity and morphological detail of whole-mount preparations. The results indicate that the most precise and structurally defined preparations were achieved with fixation at 70°C for 25 minutes. In contrast, fixation times of 20 and 40 minutes did not yield optimal results, highlighting the importance of the optimal fixation time in practical applications.

The clarity of whole-mount preparations is significantly influenced by fixation time. Notably, the highest clarity was achieved at 25 minutes, indicating that this duration allows for sufficient tissue dehydration and clearing without causing structural damage. This underscores the crucial role of fixation time, particularly 25 minutes, in achieving the highest clarity (Figure 1.). Proper fixation, mainly using a 10% KOH solution at 70°C, is essential for removing non-structural components like fats and proteins that can obstruct visibility (Azizah et al., 2022; Hidayani et al., 2018).

Morphological distinctness varied across treatments, with the 30-minute fixation resulting in the lowest clarity. In contrast, the 20- and 40-minute fixations produced moderate clarity. The highest level of morphological distinctness was observed at 25 minutes, aligning with the color quality results, where the 25-minute treatment also yielded the most vibrant color, followed by the 35-, 40-, and 20-minute treatments. The poorest color quality was again associated with the 30-minute fixation. This further confirms that 25 minutes is the optimal fixation time to achieve the best clarity, color quality, and morphological preservation, underscoring its importance in the preparation process.

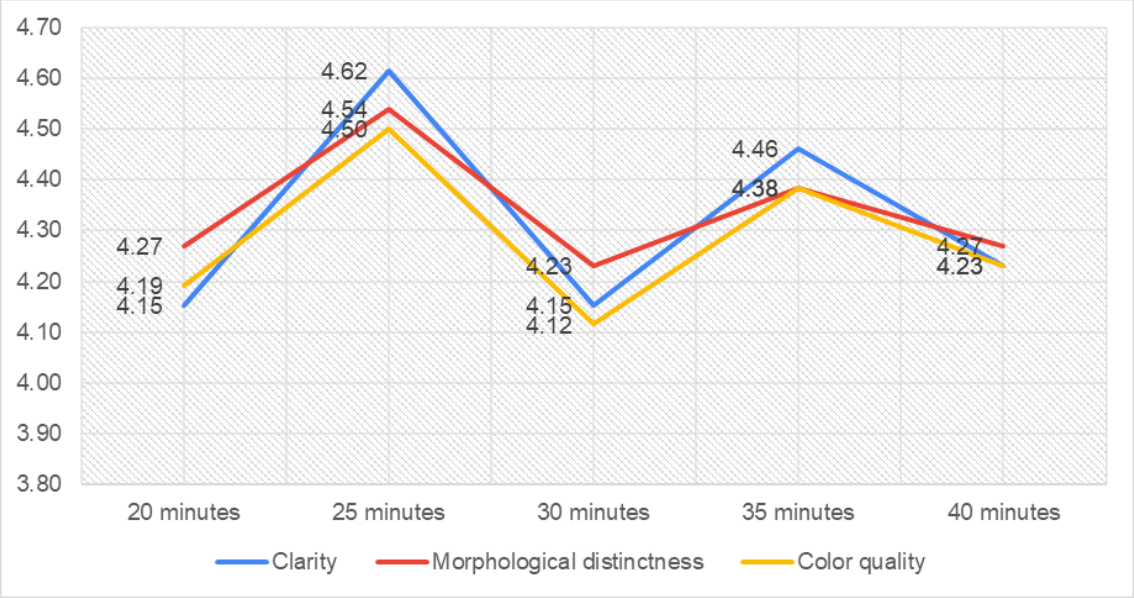

Figure 1. Diagram illustrating respondents' assessment of clarity, morphological detail, and color quality of whole mount preparations of Pediculus humanus capitis.

Fixation with 10% KOH at 70°C for 20 minutes resulted in a thick, opaque, and dark preparation, making observing the anatomical and morphological features challenging. Although the morphology appeared intact with no visible damage, the insufficient time likely did not allow the KOH to penetrate the tissue, leaving fats and proteins undissolved entirely. This led to an opaque and dark appearance, consistent with the findings of Aisyah et al. (2023), who noted that shorter KOH fixation times often result in darker specimens. Fadli et al. (2018) observed that prolonged exposure to 10% KOH, such as for 24 hours, can cause thinning of insect exoskeletons, which underscores the importance of adequate fixation. Additionally, Aisyah et al. (2023) found that toluene is more effective than xylene in the clearing process and that longer clearing times lead to more apparent specimens (Iswara & Wahyuni, 2019), highlighting the significance of the clearing process in achieving optimal results.

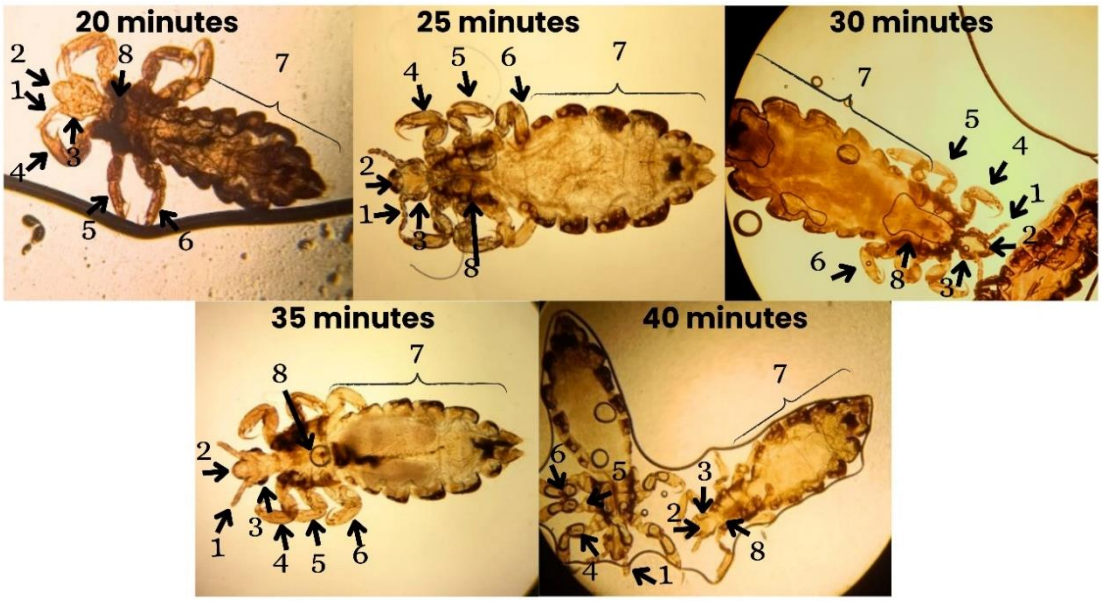

Figure 2. Photograph of Pediculus humanus capitis whole mount preparation, 1. antenna; 2. head; 3. eye; 4. proleg; 5. metaleg; 6. mesoleg; 7. Abdomen; 8. Thorax

Fixation for 25 minutes produced a clear, bright, and transparent preparation of *Pediculus humanus-capitis*, with no air bubbles and well-preserved morphological structures, including the legs, antennae, head, thorax, and abdomen. The segments between the legs were visible, and the joints connecting the thorax to the legs could be observed in detail. According to the survey results, the 25-minute fixation yielded the best preparation overall. In contrast, the 30-minute fixation produced a transparent, bright, and clear preparation, but with numerous air bubbles likely caused by insufficient pressure during the clearing stage, which allowed air to remain trapped in the specimen. Although the preparation was straightforward, the joints between the legs and thorax were not as visible, and the abdomen beneath the thorax remained dark.

Fixation for 40 minutes also produced a clear, bright, and transparent preparation, but it needed morphological completeness, as some of the metaleg and mesoleg were absent. This suggests that the prolonged fixation time caused structural damage. Karami (2012) noted that high temperatures accelerate the chemical reaction between KOH and tissue components, making the lice more transparent and facilitating morphological identification. However, excessive exposure can lead to over-fixation, as seen in the 40-minute treatment, which likely caused tissue damage and obscured morphological details. Moreover, the color quality of the 40-minute preparation was inferior to that of the 35-minute treatment, likely due to over-fixation, which can cause tissue discoloration and

degradation, as observed by Rahmawati (2011) in her study of *Ctenocephalides felis*, where prolonged KOH exposure thinned and damaged body parts. Determining the optimal fixation time is crucial to producing clear and detailed specimens. Fixation for 35 minutes at 70°C seems to strike the best balance between effective clarification and tissue preservation, ensuring minimal damage while allowing for detailed microscopic observation.

CONCLUSION

Fixation with 10% KOH at 70°C for 25 minutes was identified as the optimal condition for producing whole-mount preparations with the best clarity and morphological detail. This duration allows for effective dehydration and clarification without causing significant tissue damage. Fixation for 20 or 40 minutes did not produce optimal results, underscoring the importance of balancing fixation time and temperature. This method, which consistently delivers high-quality preparations, can be employed in various laboratory settings to ensure accurate observation and analysis.

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