Tropical Health and Medical Research
Vol. 4, No. 2, August 2022, pp. 50-57
ISSN (Online) : 2684-740X
Journal homepage:tropicalhealthandmedicalresearch.com

Utilization of Natural Dyes Solutions and Glycerol for the Quality and Durability of Direct Wet Mount Preparations Storage in Educational Laboratories

***Iswiyanti Novita, Linda Yuliana**

Medical Laboratory Technology Poltekkes Kemenkes Banjarmasin,
Mistar Cokrokusumo Street 4a Banjarbaru, Indonesia
*Email: iswiyanti10@gmail.com

Abstract: Educational laboratories require the availability of direct wet mount preparations that can last a long time with good quality. Various studies have mentioned techniques so that preparations can last a long time. This study aims to analyze the composition of dyes and glycerol in wet preparations of worm eggs on the quality and shelf life of the preparations. The solvent variations used for the wet preparations were Angkak 2%, and eosin 2%, with the addition of variations in the concentration of glycerol 20%-45%. The number of treatments and repetitions in the study was 70 samples, which were observed in the first, second, third, and fourth weeks. Based on the ANOVA test, the results showed a significant effect on the parameters measured, namely the type of dye, the type of slide, and the shelf life of the preparation (storage) in natural dye solution (2% ethanol extract of Angkak solution) and synthetic dye solution (2% eosin solution). The correlation test results of storage time treatment on the clarity of the preparations showed that the longer the storage, the lower the clarity of the preparations (r=-0.46246). The correlation test results of storage time treatment on the contrast of the preparations showed that the longer the storage, the less the contrast was not so strong (r=-0.26375). The correlation test results of storage time treatment on the quality of the preparations showed that the longer the storage, the lower the quality of the preparations (r=-0.51346). The conclusion is that the best quality of wet preparations is preparations that are stored for four weeks using a flat object-glass with a mixture of 1:1 dye solution of 2%-glycerol solution of 35% and 1:1 mixture of a 2%-glycerol solution of 1:1 solution. Further research is needed on the concentration of the dye solution mixture to find the best formulation for improving the quality and resistance of wet preparations.
Keywords: Alternative dye preparations; preserved preparations; quality of preparations

INTRODUCTION

The diagnosis of parasitic intestinal infection can be confirmed by examining feces in the parasitology laboratory using direct wet mount preparations[1]. Direct wet mount preparations are generally a reliable diagnostic method for diagnosing intestinal parasitic infections[2]. However, the direct wet mount method has lower sensitivity for detecting intestinal parasites than the fecal concentration method[3,4].

The direct wet mount method is most widely used because it is easy; rapid preparation results in direct visualization of the morphology of eggs, trophozoites, and

Corresponding Author: Iswiyanti Novita
Medical Laboratory Technology Poltekkes Kemenkes Banjarmasin.
Mistar Cokrokusumo Street 4a Banjarbaru Indonesia 70714
Email: iswiyanti10@gmail.com

parasitic cysts. The disadvantage of this method is that the preparations do not last long because they dry out in a short time. This caused the preparations could not be identified, so new preparations had to be made. The manufacture of new preparations is quite time-consuming for reagents[5].

Educational laboratories require the availability of direct wet mount preparations that can last a long time with good quality. Various studies have mentioned techniques so that preparations can last a long time. One of them is the research conducted by Asarina and Haeruni using the direct wet mount method with the addition of a 30% glycerol solution to preparations without eosin dye that can last six weeks[6]. Another study used lactophenol cotton blue (LPCB) for parasite identification[5,7]. Wet preparations made using LPCB do not dry out due to glycerol in them[8]. Solutions often used in preparing other wet stools are Saline, Quensel stain, Nairs buffered methylene blue, Lugols iodine solution, and D'Antonie's iodine stain[5].

Several studies on flora that can be used as natural dyes with eosin-like properties include red fruit juice (Pandanus sp.)[9] and soaking teak tree trunks[10]. Natural dyes from Angkak can be used as alternative dyes for wet preparations of dicotyledonous and monocot plant stems[11]. This dye is a fermented product of rice using the fungus Monascus purpures. The fungus Monascus purpureus during the fermentation process metabolizes to change the chemical composition of the rice, which causes changes in the rice because it is covered in the mycelium of the mold. The rice will then experience a color change from white to purple-red or dark red[12].

Alternative preparations with a more appropriate composition of glycerol and red yeast rice. Previous studies have shown the usefulness of glycerol in preventing dryness in wet preparations[6,8], and the findings of Angkak can be used as a natural dye[11]. However, for educational laboratory purposes, the availability of direct wet mount preparations that can last a long time with good quality is necessary. Therefore, this study aimed to analyze the composition of dyes and glycerol in wet preparations of worm eggs on the quality and shelf life of the preparations.

MATERIALS AND METHODS

The type of research used in this research is experimental research. The research time starts from August to September 2021. The research location is in the MIPA laboratory of Lambung Mangkurat University and the Parasitology laboratory of the Health Analyst Department of Health Poltekkes, Ministry of Health, Banjarmasin Indonesia.

The materials used in this study were 96% ethanol, Entellan, Aquades, Glycerol pro analysis, lens tissue, tissue, 2% ethanol extract of Angkak, and 2% eosin.

In this study, observations of the quality of temporary preparations using a microscope (Nikon) were carried out to determine the potential of a dye; both natural and synthetic, with variations in the type of glass object, the researcher used a rating scale with information as below table 1.

The criteria used in this study were the clarity of the part and the size that was caused was very clear, clear, and not clear on the observation of the worm egg part preparations. The clarity of the preparation is the clarity of the worm egg part in the preparation; this aims to distinguish the parts of the worm eggs so that they can identify the stage and species of the worm eggs.

Table 1. Interpretation of Assessment Results

Preparate Clarity		Preparate Contrasity		Preservation Preparate	
Value	Description	Value	Description	Value	Description
1	Not Clear	1	Very Contrast	1	Less
2	Clear	2	Contrast	2	Enough
3	Very Clear	3	Not Contrast	3	Good

The contrast of the preparations is the color contrast resulting from the various concentrations of coloring given to the preparations so that it can be distinguished between worm eggs and the surrounding feces. The criteria used in this study were that the colors produced were clear, clear, and unclear on the background of observing the preparations. The durability of the preparation is that the preparation should not have bubbles and dry in storage. Preparations that experience dryness will form bubbles that will interfere with observations. The dryness criteria for the preparations used in this study were to observe the wetness of the cover glass area with good criteria (76-100% wetness), sufficient (40-75% wetness), and poor (9-39% wetness) criteria.

This research was conducted by making temporary preparations (wet preparations) to examine worm eggs in feces samples[13]. Preparation of temporary preparations using the following criteria:

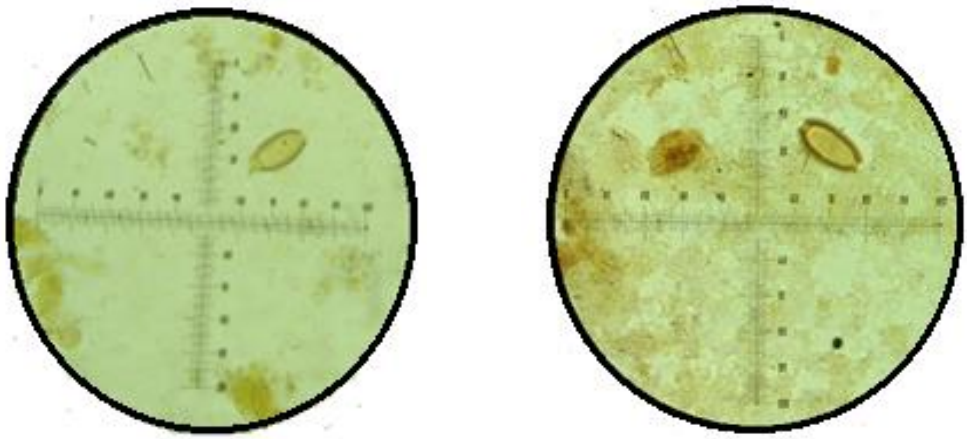
Glass object type; The types of glass objects used are glass objects/prepared glass/flat slides and glass objects/prepared glass/concave slides glass objects,

Type of dye; The types of dyes used are natural dyes (ethanol extract solution of Angkak) and synthetic dyes (eosin solution). The dye treatment is as follows:

A2G0: 2% ethanol extract solution
A2G20: a mixture of 1:1 solution of 2% ethanol extract of Angkak & 20% glycerol
A2G25: a mixture of 1:1 solution of 2% ethanol extract of Angkak & 25% glycerol
A2G30: a mixture of 1:1 solution of 2% ethanol extract of Angkak & 30% glycerol
A2G35: a mixture of 1:1 solution of 2% ethanol extract of Angkak & 35% glycerol
A2G40: a mixture of 1:1 solution of 2% ethanol extract of Angkak & 40% glycerol,
A2G45: a mixture of 1:1 solution of 2% ethanol extract of Angkak & 45% glycerol.
E2G0: 2% eosin solution
E2G20: 1:1 solution mixture of 2% eosin & 20% glycerol
E2G25: 1:1 solution mixture of 2% eosin & 25% glycerol
E2G30: 1:1 mixture of 2% eosin & 30% glycerol solution
E2G35: 1:1 solution mixture of 2% eosin & 35% glycerol
E2G40: a mixture of 1:1 solution of 2% eosin & 40% glycerol,
E2G48: a mixture of 1:1 solution of 2% eosin & 45% glycerol.

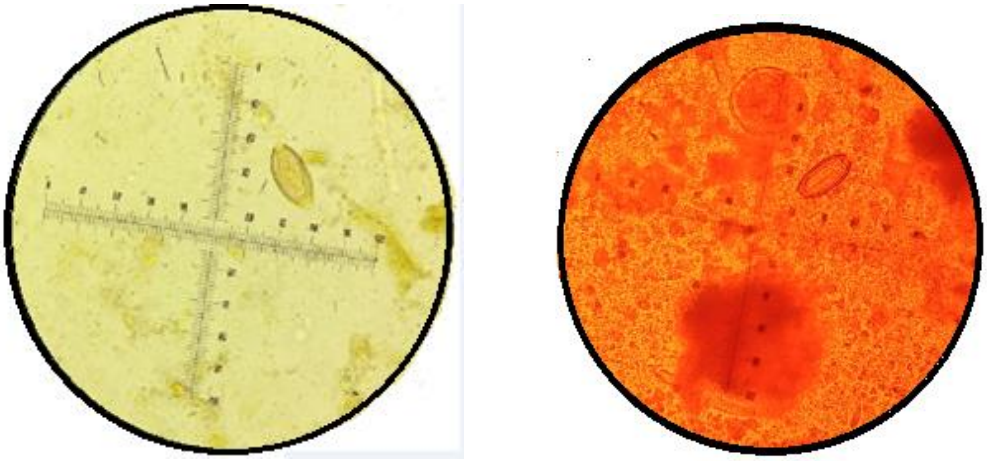
The data from observing the quality of the preparations with 70 samples for four weeks using a binocular microscope at magnifications of 100x and 400x to observe the clarity of the preparations, the contrast of the preparations, and the durability of the preparations were homogenized using =SQT(data+0.5) and followed by the Anova test and Duncan Multiple Range Test (DMRT) and Turkey's HSD correlation test.

RESULTS AND DISCUSSION



Angkak 2% + Gliserol 35% Stain Eosin 2% + Gliserol 45% Stain

Figure 1. Morphology of Trichuris trichiura Worm Eggs (400x magnification)



Angkak 2% Stain Eosin 2% Stain

Figure 1. Morphology of Trichuris trichiura Worm Eggs (400x magnification)

Table 2 shows the results of the ANOVA test, the p-value = 0.000, at the significant level the value (0.000) the value of 0.050, it shows a significant/influence on the measured parameters, namely: the type of dye has a significant effect on the quality of the preparations; the results of the Duncan Multiple Range Test (DMRT) further tests showed that the effect of the dye on the clarity of the preparations was best treated with A2G35, A2G40, and A2G45. The E2G0 treatment gave the worst preparation clarity. The effect of the dye type on the best preparations' contrast was the A2G35, A2G40, and E2G45 treatments. Treatments A2G0 and E2G0 gave the worst contrast preparations. The effect of the type of dye on the durability of the best preparations was the A2G35 and A2G45 treatments. The A2G0 treatment gave the worst preparations durability.

Tabel 2. ANOVA Results

Source	Dependent Variable	Mean Square	Sig
Corrected Model	quality of preparation clarity	.485	.000
	contrast quality of preparations	.464	.000
	quality and durability of preparations	.438	.000
Intercept	quality of preparation clarity	1509.326	.000
	contrast quality of preparations	1376.924	.000
	quality and durability of preparations	1212.251	.000
KacaObjek	quality of preparation clarity	2.803	.000
	contrast quality of preparations	2.393	.000
	quality and durability of preparations	3.107	.000
Pewarna	quality of preparation clarity	1.522	.000
	contrast quality of preparations	1.593	.000
	quality and durability of preparations	1.183	.000
Waktu	quality of preparation clarity	4.166	.000
	contrast quality of preparations	1.375	.000
	quality and durability of preparations	5.249	.000

The object-glass or glass slide is a flat transparent glass measuring 25mm x 76mm, which serves as a place to put the object to be observed under the microscope. Based on the results of ANOVA, it was shown that the type of flat object-glass significantly affected the quality of the clarity of the preparations, the contrast of the preparations, and the durability of the preparations compared to the concave object-glass. The significance of the results shown by ANOVA means that the type of flat object-glass for the durability of the preparations with the results of the quality of the clarity of the preparations and the contrast of the preparations is recommended as a direct visualization learning model (practicum) of the morphology of worm eggs in the Parasitology education laboratory.

The durability of the preparations is defined as the shelf life of 4 weeks. Shelf life has a significant effect on the quality of temporary preparations. The results of Turkey's HSD further test showed a different effect between 1 week, two weeks, three weeks, and four weeks of shelf life treatment on the quality of temporary preparations.

The correlation test results of storage time treatment on the clarity of the preparations showed that the longer the storage, the lower the clarity of the preparations (r=-0.46246). The correlation test results of storage time treatment on the contrast of the preparations showed that the longer the storage, the less the contrast was not so strong (r=-0.26375). The correlation test results of storage time treatment on the quality of the preparations showed that the longer the storage, the lower the quality of the preparations (r=-0.51346).

The results showed that the 2% red yeast rice solution had the potential to be used as a substitute for dye from the 2% eosin solution in stool examination. The results showed that the 2% red yeast rice solution gave better contrast and morphological clarity to worm eggs than the 2% eosin solution. The 2% red yeast rice solution can potentially be used as a dye for wet preparations; according to Apriani's report, that red rice red

yeast rice solution can be used as an alternative dye for wet preparations of dicotyledonous and monocotyledonous plant stems[11].

The best quality of wet preparations for examination of worm eggs observed from the clarity of preparations, the contrast of preparations, and durability of preparations (stored for four weeks) were A2G35 and A2G45 treatments. In comparison, the worst treatment is A2G0 and E2G0. The treatments A2G35 and A2G45 were 1:1 mixed treatments with 2% Angkak dye with the addition of 35% and 45% glycerol, respectively. From the appearance of the morphology of the worm eggs under the microscope, it shows that the preparations are easy to observe and clean. This is different from the eosin-glycerol mixture dye preparations, which show a reddish color, making it more challenging to observe. These results are consistent with a study conducted by Asarina and Haeruni, which stated that adding a 30% glycerol solution to preparations without eosin dye lasted six weeks[6].

Other studies have also shown the ability of glycerol to make preparations last without drying out[8]. Research by Vignesh et al. 2008 showed that wet preparations with the iodine-glycerol solution could clearly distinguish the parasite's internal structure and provide accurate identification. Iodine-glycerol solution preparations last longer without drying up to 2 days, whereas iodine-glycerol solution preparations dry in <10 minutes[8]. Glycerol or glycerin ($C_3H_8O_3$) is a viscous, colorless, odorless, clear hygroscopic liquid. Color quality is influenced by anthocyanin stability, pH, temperature, light, and the presence of metal ions[14]. Glycerol functions as a preservative, solvent, and humectant[15]. Research by Khanna V and colleagues stated that the addition of glycerol functions as a preservative because it has hygroscopic properties so that it can absorb water from the surrounding environment and prevent drying of wet preparations for examining worm eggs[16].

The limitation of this research is the lack of variation in the concentration of the natural dyes (angkak) used. This study's results recommend using a 2%-glycerol 35% red yeast solution for the best quality and durability of wet preparations of worm eggs.

CONCLUSION

There are differences in the results of observations on the parameters of the type of dye, the type of object-glass, and the durability of the preparations (storage) in natural dye solutions (2% ethanol extract solution of Angkak) and synthetic dye solution (2% eosin solution). The best quality occurred in wet preparations for examining worm eggs which were stored for four weeks using a flat slide with a 1:1 mixture of 2% glycerol solution and 1:1 mixture of 2% glycerol solution and 1:1 mixture of 2% glycerol solution. The use of flat object-glass gives better results than concave glass objects. Further research is needed on the concentration of the dye solution mixture to find the best formulation for improving the quality and resistance of wet preparations.

ACKNOWLEDGEMENT

Acknowledgments are conveyed to appropriate parties, especially to the institution or person assisting the research, for example: to the donor, facilities, materials, or advice. Do not give thanks to one of the authors.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

REFERENCE

1. Parija, S.C. and Srinivasa, H. Viewpoint: the neglect of stool microscopy for intestinal parasites and possible solutions. Trop. Med. and Inter. Health, 1999; 4: 522-524.
2. Utzinger J, Rinaldi L, Lohourignon LK, et al. FLOTAC: a new sensitive technique for diagnosing hookworm infections in humans. Trans R Soc Trop Med Hyg, 2008; 102(1): 84–90. doi: 10.1016/j.trstmh.2007.09.009
3. Demeke G, Fenta A and Dilnessa T. Evaluation of Wet Mount and Concentration Techniques of Stool Examination for Intestinal Parasites Identification at Debre Markos Comprehensive Specialized Hospital, Ethiopia, 2021; 14: 1357–1362. doi: 10.2147/IDR.S307683
4. Hailu T, Abera B. Performance evaluation of direct saline stool microscopy, Formol ether concentration and Kato Katz diagnostic methods for intestinal parasitosis in the absence of gold standard methods. Trop Doct, 2015; 45(3): 178–182. doi: 10.1177/0049475515581127
5. Parija SC, Prabhakar PK. Evaluation of lactophenol cotton blue for wet mount preparation of feces. Journal of clinical microbiology, 1995 Apr; 33(4): 1019-21.
6. Asarina S, Haeruni N. Evaluasi Penggunaan Gliserol dalam Pembuatan Preparat Telur Cacing Semipermanen. Jurnal Pengelolaan Laboratorium Pendidikan, 2019 Jul 17; 1(2): 37-40.
7. Parija, S.C. Laboratory diagnosis of parasitic infestations. National Med. J of India, 1993; 6: 283-289
8. Vignesh R, Sekar R, Shankar EM, Kumarasamy N, Murugavel KG, Irene P, Solomon S, Balakrishnan P. Wet mounting using iodine–glycerol provides a semi-permanent preparation for microscopic observation of fecal parasites. Journal of Medical Microbiology, 2008; 57(5): 679-680. DOI 10.1099/jmm.0.47851-0
9. Oktari A, Muâ A. Optimasi air perasan buah merah (Pandanus sp.) pada pemeriksaan telur cacing. Jurnal teknologi laboratorium, 2017 Jul 8; 6(1): 8-17.
10. Sari, Sispita Y, Artanti D, Roz F. Optimalisasi Rendaman Batang Kayu Pohon Jati (*Tektona Grandis*). Teklabmed Jurnal Teknologi Laboratorium Medik, 2020; 1(1).
11. Apriani I. Pengembangan media belajar: angkak beras merah dan teh (Camellia sinensis) sebagai pewarna alternatif preparat basah jaringan tumbuhan. Bioilmi: Jurnal Pendidikan, 2016 Jun 28; 2(1).
12. Atma Y. Studi penggunaan angkak sebagai pewarna alami dalam pengolahan sosis daging sapi. Jurnal Teknologi, 2015 Jul 30; 7(2): 76-85.
13. Natadisastra D. Penuntun Praktikum Ilmu Parasit (Protozologi) untuk Fakultas Kedokteran Universitas Padjajaran. FK. Unpad: Bagian Parasitologi. 2009.
14. Sophia AS. Optimasi air perasan ubi jalar ungu (*Ipomea batatas* L.) pada pemeriksaan telur cacing. Bioma: Jurnal Biologi Makassar, 2022 Jun 6; 7(2): 8-13.
15. Mirzayanti YW. Pemurnian gliserol dari proses transesterifikasi minyak jarak dengan katalis sodium hidroksida. In Seminar Nasional & Teknologi Terapan. Jurusan Teknik Kimia, Institut Teknologi Adhi Tama Surabaya, 2013 Apr: 267-273.

16. Khanna V, Tilak K, Rasheed S, Mukhopadhyay C. Identifying and preserving intestinal parasites using methylene blue-glycerol mount: a new approach to stool microscopy. Journal of Parasitology Research. 2014 Apr 10: 2014.