



A highly-effective modified hard tick (*Ixodes*)-slide preparation method using lactophenol in comparison with a daily basis-used potassium hydroxide technique

Amal Hasan Atiyah

Middle Technical University, Institute of Medical Technology/Baghdad, Iraq

Abstract

The conducted work, here, was aimed at using a lactophenol-based technique, a method for roundworm slide preparation, in the slide preparation of hard ticks (*Ixodes*) and compared its outcomes with those by a KOH-based technique, which can generate black-colored appearance of the ticks in the prepared slides due to the burning effect of KOH. Here, ticks were placed in the lactophenol solution (1 part of lactic acid, 1 part of phenol, 2 parts of glycerin, and 1 part of distilled water) for one to three months, depending on the thickness of ticks, at room temperature (22-30°C). For the KOH method, 70% alcohol was used immediately after placing the ticks in the KOH solution. The results of the lactophenol method showed high quality and very clear tick slides when compared with those prepared by the use of KOH, which revealed dark-looking tick appearance. The current study provides an excellent technique that involves the utilization of lactophenol to prepare high quality tick slides for better diagnosis and scientific studies of hard ticks (*Ixodes*).

Keywords: hard ticks, ixodes, koh, lactic acid, lactophenol, phenol

Introduction

Insects, chiggers, mites, and ticks are just few of the arthropods that blood-feast on terrestrial vertebrates throughout their lives. A number of ticks (*Acari*, *Ixodidae*) are must-hematophagous ticks. Because of their parasitic nature, they are destined to spread microparasites that may be harmful to people and domestic animals. Ixodid tick-borne pathogens include viruses, protozoans, bacteria, and nematodes (Kahl, 2018) [7].

An important category of arthropods is those that carry diseases like ticks. Ticks may transmit a number of critical pathogens. Many of the illnesses carried by ticks are exclusive to a particular genus or species. Because of this, many clinical environments need the clinical diagnostic laboratories to be able to recognize ticks to the genus or, better still, to the species rank. As far as public health is concerned, there are just two families of ticks (*Ixodidae*, the hard ticks, and *Argasidae*, the soft ticks) (Rochlin *et al.*, 2013) [11]. Both *Ixodidae* and *Argasidae* may be distinguished by the existence or lack, respectively, of a dorsal shield (scutum). The mouthparts (capitulum) of ixodid ticks may be seen from above, whereas the mouthparts of argasid ticks are concealed from above. Screening labs often get ixodid ticks because of the length of time they stay attached to the animal. On contrary, argasid ticks eat intermittently and do not stay attached to their host for long periods of time. Because of this, they can only be sent to the lab on a very limited basis (Mathison & Pritt, 2014) [10].

The detection of the hard ticks at the genus or species level may require specific treatment applied to the tested ticks, if a tick is mounted, such as the use of KOH for clearing purposes and followed by dehydrating process via the utilization of alcohol. This can specifically beneficial for the detection of the tick young stages; larvae, where the hair-like structures (body setae) are critical for the taxonomical studies (Balasubramanian *et al.*, 2019) [1]. This method can generate low quality slides with dark-appearing ticks on the prepared slides, and this is due to the burning effect of the potassium hydroxide on the tested tick. Thus, the conducted work, here, was aimed at using a lactophenol-based technique, a method for roundworm slide preparation, in the slide preparation of hard ticks (*Ixodes*) and compared its outcomes with those by a KOH-based technique for the purpose of better slide preparation.

Materials and methods

Ticks

Ticks were collected using clean a forceps and placed in clean containers. The samples were then transported to the Laboratory of Parasitology, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah City, Iraq.

Lactophenol method

In the Lab, ticks were placed in the lactophenol solution (1 part of lactic acid, 1 part of phenol, 2 parts of glycerin, and 1 part of distilled water) in glass tubes for one to three months, depending on the thickness of ticks, at room temperature (22-30°C). After that, these specimens were ready to mount with Dpx or canadabalsam.

Then, the slides were placed in a warm oven or on a hot plate (45°C) for about two days. After that, the slides were ready for microscopic investigation.

KOH method

For the KOH method, 70% alcohol was used immediately after placing the ticks in the KOH solution. The method was followed from a method described by (L. Garcia, 2007; L. S. Garcia & Procop, 2016) [7, 5].

Results

The results of the lactophenol method showed high quality and very clear tick slides (Figure 1) when compared with those prepared by the use of KOH (Figure 2), which revealed dark-looking tick appearance.



Fig 1: Images of hard ticks (Ixodid) prepared by lactophenol method



Fig 2: Images of hard ticks (Ixodid) prepared by KOH method.

Discussion

Ticks are important arthropod that are considered as important disease vector of many pathogens, such as viruses, bacteria, and protozoa. This importance makes their identification to the genus or even the species level critical for diagnostic purposes and/or entomological studies. This identification process faces challenges, especially when investigating the tick external body features via both macroscopic and microscopic examination. One of these obstacles is the use of KOH in clearing a tick for slide preparation that can generate low quality slides due to the burning effect of KOH left on the tested ticks (Lodha & Poojary, 2015) ^[9].

The present work showed important findings, in which the lactophenol method gave highly clear tick slides that can be visualized easily, showing the distinguishing body structures of the tested ticks. The lactophenol method generally is used in slide preparation of nematodes (Zahabiun *et al.*, 2015) ^[13]. Zahabiun *et al.* (Zahabiun *et al.*, 2015) ^[13] reported that the use of lactophenol prevents any nematode-related structural damages, which may occur due to the use of alcohol-based dehydration processes in other methods, such as shrinkage of the treated worms. Moreover, the authors recorded that the nematode shapes and integrity are kept intact for long period of time when using the lactophenol (Zahabiun *et al.*, 2015) ^[13].

The lactophenol method also showed successful slide preparation of mites (Mathison & Pritt, 2014) ^[10], which agrees with the current study findings that indicates the positive results in the slide mount of ticks tested in the present study. One of the important genera of hard ticks is *Hyalomma* spp., which is considered a worldwide-spread pathogen-transmitting vector that causes the transmission of several important pathogens. There are similar morphological features between *Hyalomma* spp. and *Amblyomma* spp. but the dorsal shield, forecoxae, and anal plates in the males can be used for better differentiation between the two genera (Chitimia-Dobler *et al.*, 2019; Choubdar *et al.*, 2019; Gharbi & Darghouth, 2014; Kumar *et al.*, 2020; Spengler & Estrada-Peña, 2018) ^[2, 3, 6, 8, 12]. So, the use of a good slide-mounting solution is important to have high quality tick slides that can be visualized easily to detect these distinct body features.

Conclusion

The current study provides an excellent technique that involves the utilization of lactophenol to prepare high quality tick slides for better diagnosis and scientific studies of hard ticks (Ixodes).

References

- Balasubramanian R, Yadav PD, Sahina S, Arathy Nadh V. Distribution and prevalence of ticks on livestock population in endemic area of Kyasanur forest disease in Western Ghats of Kerala, South India. *Journal of Parasitic Diseases: Official Organ of the Indian Society for Parasitology*,2019;43(2):256-262. <https://doi.org/10.1007/S12639-019-01086-7>
- Chitimia-Dobler L, Schaper S, Rieß R, Bitterwolf K, Frangoulidis D, Bestehorn M *et al.* Imported *Hyalomma* ticks in Germany in 2018. *Parasites & Vectors*,2019;12(1):1-9. <https://doi.org/10.1186/S13071-019-3380-4>
- Choubdar N, Oshaghi MA, Rafinejad J, Pourmand MR, Maleki-Ravasan N, Salehi-Vaziri M *et al.* Effect of Meteorological Factors on *Hyalomma* Species Composition and Their Host Preference, Seasonal Prevalence and Infection Status to Crimean-Congo Haemorrhagic Fever in Iran. *Journal of Arthropod-Borne Diseases*,2019;13(3):268-283. [/pmc/articles/PMC6928388/](https://pubmed.ncbi.nlm.nih.gov/36928388/)
- Garcia L. *Diagnostic medical parasitology* (5th ed.). ASM Press, 2007.
- Garcia LS, Procop GW. Diagnostic Medical Parasitology. In A. L. Truant, Y. Tang, K. B. Waites, C. Bébear, & R. P. Rennie (Eds.), *Manual of Commercial Methods in Clinical Microbiology* (1st ed,2016:1:284-308. John Wiley & Sons, Ltd. <https://doi.org/10.1002/9781119021872.CH15>
- Gharbi M, Darghouth MA. A review of *Hyalomma scupense* (Acari, Ixodidae) in the Maghreb region: from biology to control. *Parasite*,2014;21(2):112. <https://doi.org/10.1051/parasite/2014002>
- Kahl O. Hard ticks as vectors—some basic issues. *Wiener Klinische Wochenschrift*,2018;130(15):479-483. <https://doi.org/10.1007/S00508-018-1360-X>
- Kumar B, Manjunathachar HV, Ghosh S. A review on *Hyalomma* species infestations on human and animals and progress on management strategies. *Heliyon*,2020;6(12):e05675. <https://doi.org/10.1016/J.HELIYON.2020.E05675>
- Lodha N, Poojary SA. A Novel Contrast Stain for the Rapid Diagnosis of Pityriasis Versicolor: A Comparison of Chicago Sky Blue 6B Stain, Potassium Hydroxide Mount and Culture. *Indian Journal of Dermatology*,2015;60(4):340-344. <https://doi.org/10.4103/0019-5154.160476>
- Mathison BA, Pritt BS. Laboratory Identification of Arthropod Ectoparasites. *Clinical Microbiology Reviews*,2014;27(1):48-67. <https://doi.org/10.1128/CMR.00008-13>
- Rochlin I, Ninivaggi DV, Hutchinson ML, Farajollahi A. Climate change and range expansion of the Asian tiger mosquito (*Aedes albopictus*) in Northeastern USA: implications for public health practitioners. *PLoS One*,2013;8(4):e60874. <https://doi.org/10.1371/JOURNAL.PONE.0060874>
- Spengler JR, Estrada-Peña A. Host preferences support the prominent role of *Hyalomma* ticks in the ecology of Crimean-Congo hemorrhagic fever. *PLoS Neglected Tropical Diseases*,2018;12(2):e0006248. <https://doi.org/10.1371/JOURNAL.PNTD.0006248>
- Zahabiun F, SADJJADI SM, ESFANDIARI F. Development of a Double Glass Mounting Method Using Formaldehyde Alcohol Azocarmine Lactophenol (FAAL) and its Evaluation for Permanent Mounting of Small Nematodes. *Iranian Journal of Parasitology*,2015;10(4):617-624. [/pmc/articles/PMC4724839/](https://pubmed.ncbi.nlm.nih.gov/304724839/)