



Testing for duck hepatitis B virus in ducks using loop-mediated isothermal amplification



Veronica Clark

School of Science and Medicine
Lake Superior State University

Abstract

Hepatitis B virus is a very common infection among humans, infecting up to 80,000 Americans each year. Viruses that are very closely related to human hepatitis are found among other species such as woodchucks (woodchuck hepatitis virus) and ducks (duck hepatitis B virus). This close relation among these viruses has made it possible to use these animals as models to study hepatitis B in vitro, especially in ducks. Duck hepatitis B virus is often studied in domesticated ducks or ducks used for science, but wild ducks have not been actively tested for infection likely due to showing little to no signs of illness when infected. This study tested for duck hepatitis B virus in a sample of wild ducks harvested throughout the hunting season. To determine the presence of the virus, DNA from the livers of 26 ducks was extracted and exposed to Loop-mediated Isothermal Amplification (LAMP). This method of amplification used a colorimetric reaction to display a result. No color change was observed, thus signifying zero samples were positive for duck hepatitis B virus. This study was conducted in the lab, but demonstrated LAMP is a feasible method to be used in the field due to its requirement of less resources and time and its generally wide temperature range.

Background

It is a relevant concern for humans to have about the presence, transmission, and persistence of the hepatitis B virus. The study of duck hepatitis B virus, in terms of human health and how the virus operates, has made progress toward a better understanding of HBV in humans. The biological and structural properties of the two viruses are comparable on a large scale (Murray et al. 1991). Although, human HBV may lead to illness if left untreated while ducks show little to no signs of infection at all. With that being said, they can still have prolonged infection (Ji et al. 2019). Most of the information about duck hepatitis B virus has been obtained by testing domestic ducks used for science or meat production. There is not much information on the impact this virus has on wild populations. While the health of ducks do not seem to be seriously affected by infection, the virus should still be monitored to obtain any information valuable to understanding it.

Objective

The goal of this study was to determine if duck hepatitis B virus infects wild populations of ducks, and if so, determine how prevalent it is in Michigan's Upper Peninsula.

Methods

- Ducks of various species collected from local hunters
- Livers removed and kept with corresponding wing
- Kept frozen at -20°C
- Liver samples washed in phosphate-buffered saline (PBS)
- Mortar and pestle used to grind liver into paste
- Liquid nitrogen for cell lysis
- Added to tube with PBS
- Frozen and thawed three times
- DNA extracted using EasyPure Viral DNA/RNA Kit from TransGen Biotechnology Inc.
- Extraction completed by using a spin column
- DNA amplified using Loop-mediated Isothermal Amplification (LAMP) (Figure 1).
- LAMP material provided by New England Biolabs Inc.
- Colorimetric master mix used to display positive or negative result

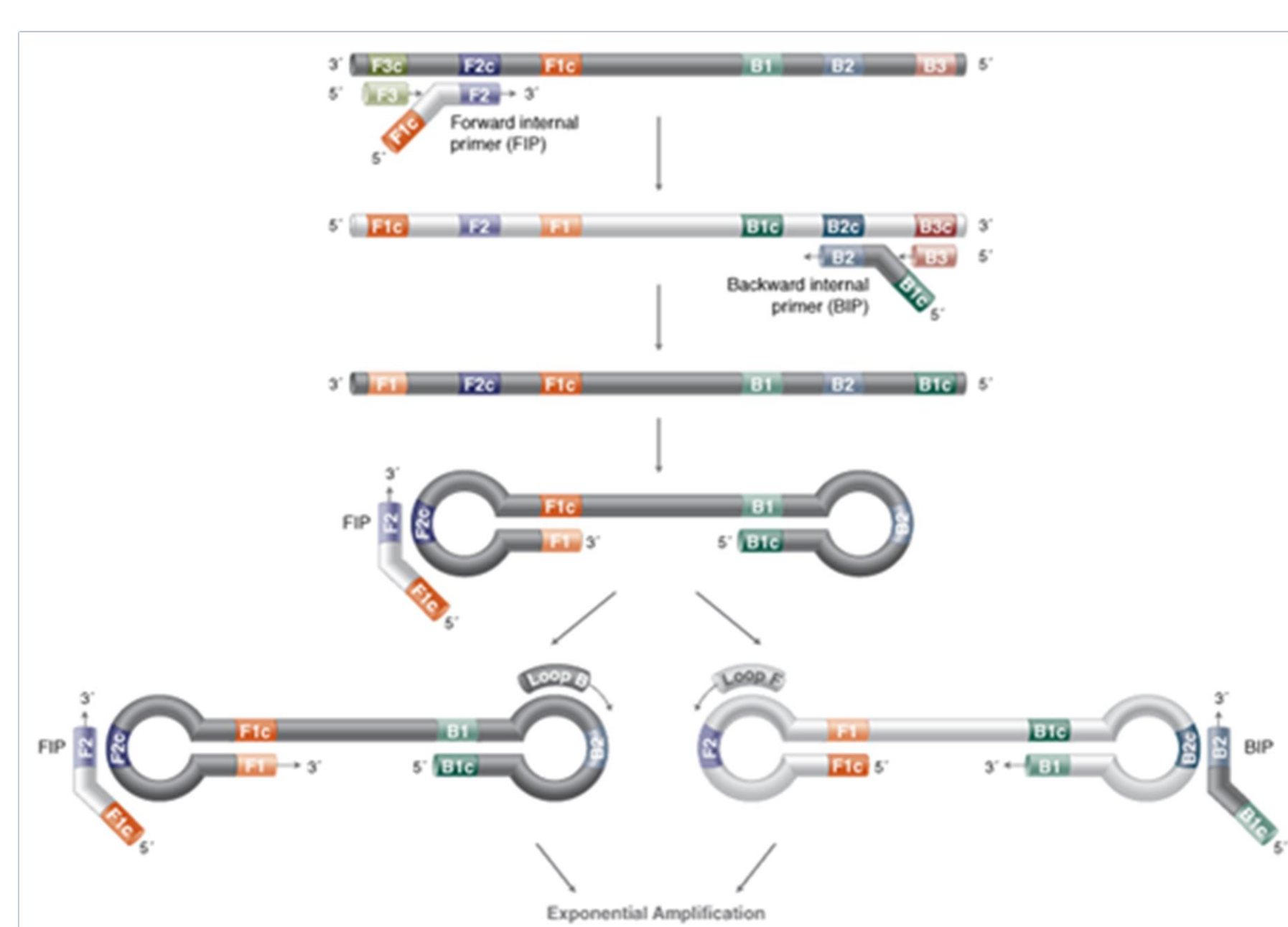


Figure 1. Diagram showing the process of loop-mediated isothermal amplification. A strand-displacing DNA polymerase starts synthesis and the primers form loop structures to promote the amplification.

Breed	Total
GWTE	4
MALL	8
AMWI	3
WODU	1
BUFF	3
COGO	2
LTDU	1
LESC	2
RNDU	2

Figure 2. Table showing the totals of each duck species tested for duck hepatitis B virus in this project.

Results

The harvest collection from Michigan's Eastern Upper Peninsula yielded 26 ducks of nine species (Figure 2). No abnormalities were visibly present in any of the ducks tested. Out of the 26 DNA samples tested through loop-mediated isothermal amplification, all colorimetric results remained pink (Figure 3). This means that zero of the ducks tested positive for DHBV (Figure 4).

Discussion

Loop-mediated isothermal amplification (LAMP) was used to test 26 wild ducks from Michigan's Eastern Upper Peninsula. The results of this experiment lead to zero positive samples. This does not mean that DHBV does not affect wild duck populations, due to several factors to be considered. In this particular case, the sample size may have been too small for such a wide-spread taxon. Increasing the sample size would also increase the chances of a positive test. For the experiment, a control was not present due to lack of ability to purchase DNA positive for DHBV. A positive control would have been helpful in verifying the accuracy of the test method used on each sample. Another factor to be considered is the time between harvest and DNA extraction. It is possible that ducks were harvested in the early hours of the day and collected hours later, which may have caused decreased detectability of the virus. To remedy this in future experiments, the shorter requirement of time, resources, and a generally wide temperature range makes the LAMP method reliable to use directly in the field (Ji et al. 2019).

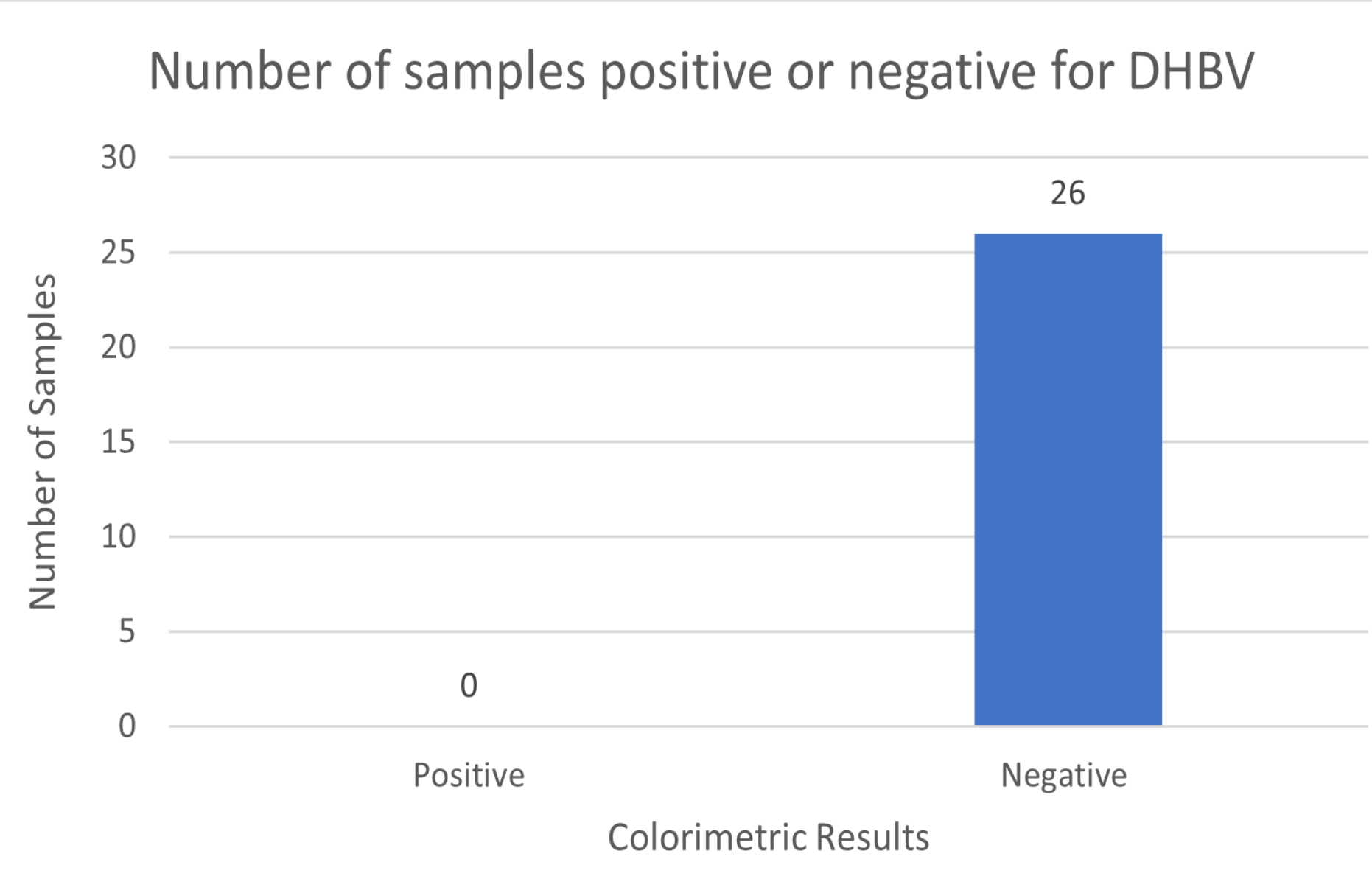


Figure 3. Bar graph showing the results of LAMP and colorimetric analysis to show zero positive results among the 26 total ducks tested for duck hepatitis B virus (DHBV).

Acknowledgements

I would like to thank Dr. Jason Garvon for his continued support and assistance throughout the duration of this project. I would also like to thank the local waterfowl hunters for the donation of ducks, especially Andrew Kaiser, Zack Walters, and friends. I would also like to thank Dr. Jun Li and Dr. Stephen Kolomyjec for their input and assistance in making this project practical. Thank you to all my friends and family for all the extra support and encouragement.

Literature Cited

- Hepatitis B Foundation. What is hepatitis B? Retrieved on Feb. 24, 2020 from <<https://www.hepb.org/what-is-hepatitis-b/what-is-hepb/facts-and-figures/>>.
- Ji, J., Xu, X., Wu, Q., Wang, X., Li, W., Yao, L., Kan, Y., Yuan, L., Bi, Y., and Xie, Q. 2019. Simple and visible detection of hepatitis B virus in ducks and geese using loop-mediated isothermal amplification. *Poultry Science* 99: 791-796.
- Murray, S.M., J.S. Freiman, K. Vickery, D. Lim, Y.E. Cossart and R.K. Whiteley. 1991. Duck hepatitis B virus: a model to assess efficacy of disinfectants against hepadnavirus infectivity. *Epidemiology and Infection* 106(3): 435-443.
- Ryu, W.S. 2017. Hepadnaviruses. *Molecular Virology of Human Pathogenic Viruses*. 247-260.

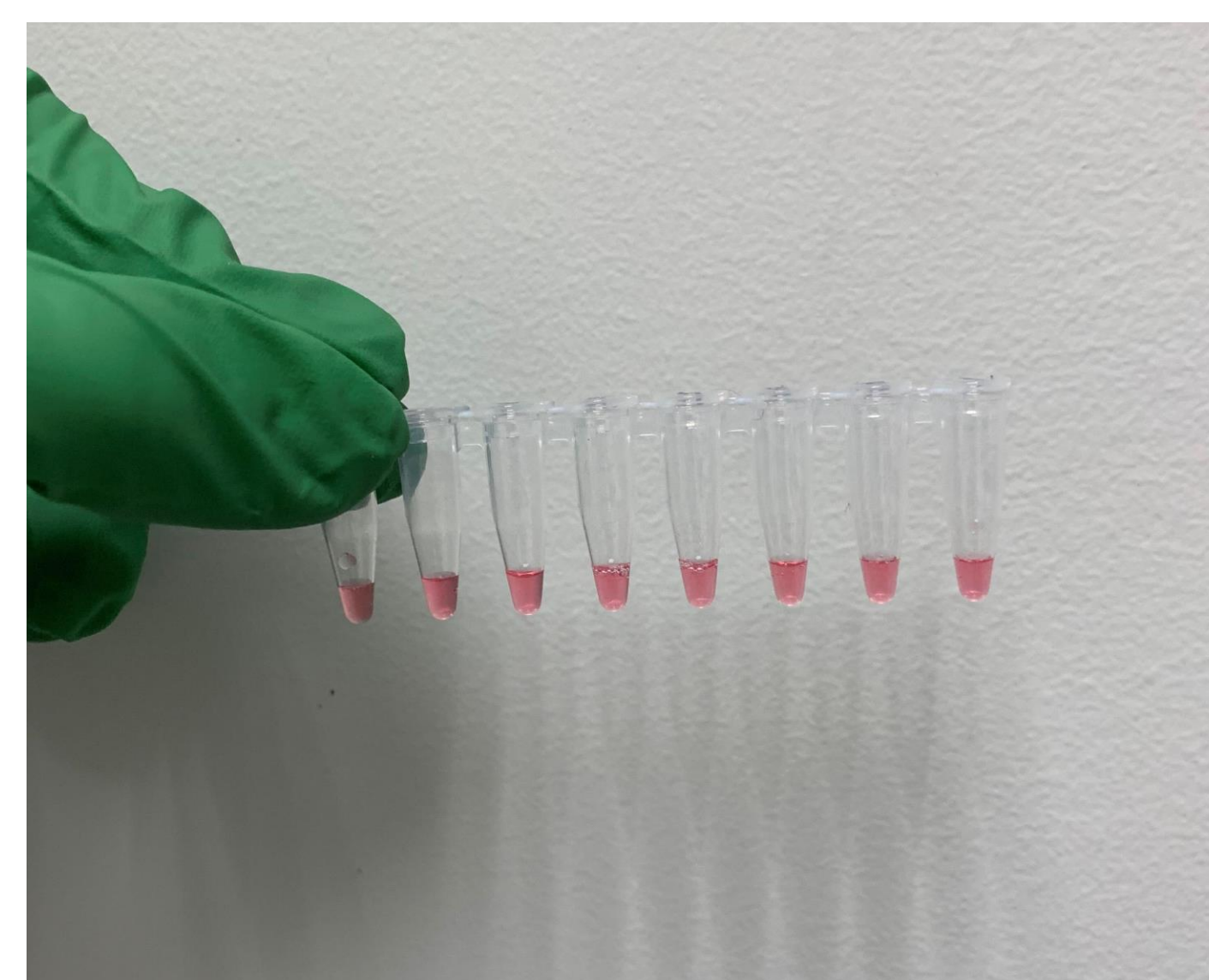


Figure 4. Results of LAMP and colorimetric analysis showing only pink coloration (negative), implying no presence of DHBV in the samples.