

Rapid Test for Detection of Hepatitis B Virus in Human Breastmilk

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(Received: 25 February 2016; accepted: 18 April 2016)

Hepatitis B viral infection is a significant health problem that can be transferred from mothers to babies during birth or breastfeeding. Breastmilk that is donated to breastmilk banks or directly mother to mother needs to be tested to make sure it is not infected with hepatitis B to ensure it is safe to give to babies. This work showed that it was possible to detect the presence of hepatitis B in breastmilk after centrifugation rather than having to use a blood sample. In addition, a five minute exposure to a temperature of 72°C was shown to be able to kill the virus in the breastmilk. The proposed method has the potential to shorten the time needed to process milk donations and make the screening less complicated.

Keywords: Breastmilk bank, mother to mother donation, HBV, screening.

Infection with hepatitis B virus (HBV) is a major health problem globally, and it results in acute or chronic hepatitis B as well as cirrhosis of the liver or hepatocellular carcinoma.¹ The HBV can be transmitted from the mother to the child during birth or subsequent breastfeeding,^{1,2} but it is possible that with suitable precautions it will be possible to prevent the vertical infection of infants from their mothers.³

There is a consensus that breastmilk is the best option for feeding newborn babies.⁴ However, not all mothers are able to provide their newborn children with breastmilk, either due to insufficient production or not being present, or

where the mother's breastmilk might be harmful.⁵ In such situations it is best for the infant to be provided with breastmilk from a breastmilk bank. These are services generally administered by hospitals that collect breastmilk from mothers and then screen, process, store and distribute it to the infants that require it.⁶ Another alternative is for direct donation between mothers where a breastmilk bank is not available.

It is vitally important that all the breastmilk provided, either by a breastmilk bank or directly, is safe to consume. This means that it should be free from infectious agents so it does not transmit diseases to the babies. Currently, blood tests are performed to determine whether the donating mothers are HBV positive.⁷ In addition, breastmilk is screened for a selection of the following diseases: human immunodeficiency

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virus (HIV), hepatitis C human T-cell leukemia virus (HTLV), tuberculosis and syphilis.^{4,6} Despite a suggestion that pasteurization of donated breastmilk was not needed,⁸ it is now recommended for all donated milk at 62.5°C for 30 minutes.^{5,7}

There are two main drawbacks with the current process of milk donation related to HBV infection. The first is that a blood sample is required to determine whether the donating mother is infected with HBV, and the second is that the 30 minute pasteurization step is quite time consuming. The aim of the current work was to initially determine whether it was possible to detect the HBV in the milk of a donating mother, thereby eliminating the need for a blood test, and to reduce the time needed for pasteurization.

MATERIALS AND METHODS

Blood and breast milk samples were obtained from a HBV infected mother. The milk was collected with a breast pump and stored at 4°C

for one day before testing. The blood sample (5 ml) was taken from the brachial vein. The blood sample was left in a tube in a standing position at room temperature for two hours to allow a clot to form, and then the clot was spun down by centrifugation at 3,400 rpm for three minutes, after which the serum was taken. The serum was stored at 4°C for one day before testing.

The serum was removed from the fridge and left to stand at room temperature for 10 minutes. Then 100 µl of the serum was dropped onto a HBsAg test kit (Standard Diagnostics, Inc., Korea) that was used to detect the presence of HBV to confirm that the mother was HBV infected.

A breast milk sample was left to stand at room temperature for two hours: until the fat layer had separated leaving a clear layer. Then 20 µl of the clear layer was dropped onto the well on a HBsAg test kit. Two further breast milk samples were separated by centrifugation at 5,000 or 10,000 rpm for three minutes, and the clear layer was tested as previously described.

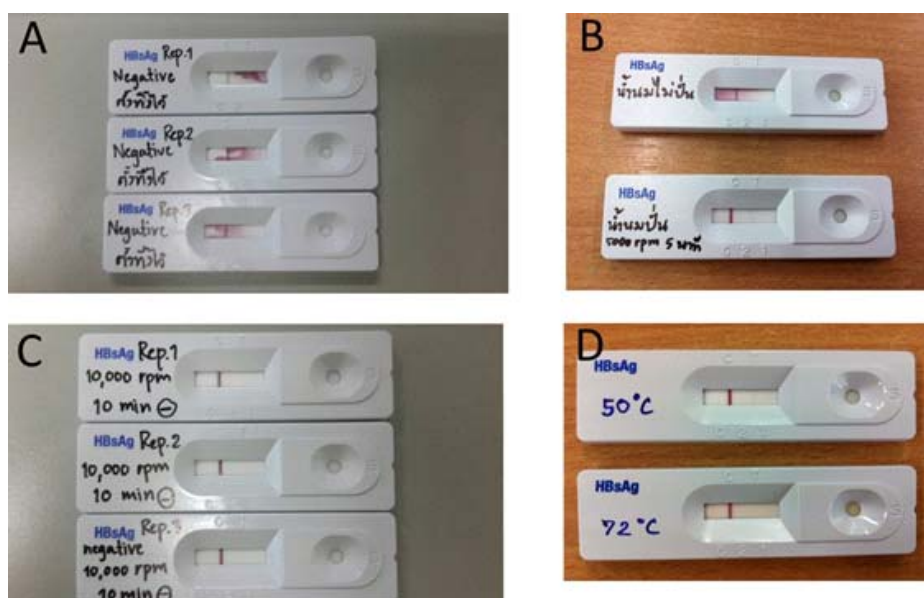


Fig. 1. A) Hepatitis B surface antigen was negative from clear layer of breastmilk after being left to stand at room temperature for two hours. B) Hepatitis B virus detection in breastmilk: hepatitis B surface antigen was negative for breastmilk without centrifugation (upper) but positive for breastmilk with 5,000 rpm centrifugation (lower). C) Hepatitis B virus detection in breastmilk: hepatitis B surface antigen was negative for breastmilk with 10,000 rpm centrifugation. D) Hepatitis B virus detection in breastmilk: hepatitis B surface antigen was positive after incubating breastmilk at 50°C for five minutes. Hepatitis B surface antigen was negative after incubating breastmilk at 72°C for five minutes

Finally, two breast milk samples were heated to either 50 or 72°C for five minutes, left for five minutes at room temperature and then centrifuged at 5,000 rpm for three minutes. The clear layer was tested for the presence of HBsAg as described previously.

RESULTS AND DISCUSSION

A traditional blood test confirmed that the milk donor was HBV infected.

The breastmilk sample that was left to separate at room temperature gave a negative result from the HBsAg test kit (Fig. 1A). However, when the sample was separated into fat and clear layers using a centrifuge at 5,000 and 10,000 rpm, it was found that the 5,000 rpm sample showed a positive result (Fig. 1B) while the 10,000 rpm one was negative (Fig. 1C). This shows that a centrifugation step is required to obtain a suitable separation of the sample, but that a suitable speed has to be selected, as if it is too great it will prevent the test from giving a true result.

The sample that was heated to 50°C for five minutes and then centrifuged at 5,000 rpm gave a positive result (Fig. 1D), which indicated that the temperature and time were not suitable to kill the HBV and make the milk safe. However, when the breastmilk was heated to 72°C for five minutes before being separated at 5,000 rpm and tested, the result was negative (Fig. 1D), thereby showing that the short hot pasteurization step had made the milk safe to consume.

This work further supports the possibility of using shorter and hotter heat treatments than the current standard for the preparation of donated milk.⁵ This would make the process of ensuring the donated breastmilk was safe to use faster and more convenient.

In addition, the ability to detect the presence of the HBV in breastmilk has several benefits: firstly, it is a rapid detection process that is easy to perform; secondly, no blood samples are required, just the milk sample; and thirdly, the only

equipment required is a centrifuge. This means that the method has the potential to be ideal for the rapid screening of milk being donated between mothers and to breastmilk banks. Further work is now required to test the method with a larger sample size.

ACKNOWLEDGMENTS

The work was supported by Mahasarakham University.

REFERENCES

1. Wang, T., Wang, M., Duan, G., Chen, X., and He, Y. Discrepancy in impact of maternal milk on vertical transmission between hepatitis B virus and human cytomegalovirus. *International Journal of Infectious Diseases*, 2015; **37**: e1-e5.
2. Lin, H., Hsu, H., Chang, M., Chen, P., and Chen, D.. Hepatitis B virus in the colostrum of HBsAg-positive carrier mothers. *Journal of Pediatric Gastroenterology and Nutrition*, 1993; **17**: 207-210.
3. Choe, B. A disappearing vertical infection: Will hepatitis B be a forgotten disease in children? *Korean Journal of Internal Medicine*, 2014; **29**: 296-300.
4. Yadav, S., and Rawal, G. Human breast milk bank. *International Journal of Health Science and Research*, 2015; **5**(6): 592-597.
5. Bharadva, K., Tiwari, S., Mishra, S., Mukhopadhyay, K., Yadav, B., Agarwal, R. K., and Kumar, V. Human milk banking guidelines. *Indian Pediatrics*, 2014; **51**: 469-474.
6. Bhatnager, M., Kapil, U., and Gujral, H. Human milk banks and their relevance in India. *The Indian Journal of Pediatrics*, 2013; **81**: 1061.
7. Baumer, J. H. Guidelines for the establishment and operation of human milk banks in the UK. *Archives of Disease in Childhood Education and Practice*, 2004; **89**: ep27-ep28.
8. Bjorksten, B., Burman, L. G., Chateau, P. D., Fredrikzon, B., Gothefors, L., and Hernell, O. Collecting and banking human milk: To heat or not to heat? *British Medical Journal*, 1980; **281**: 765-769.