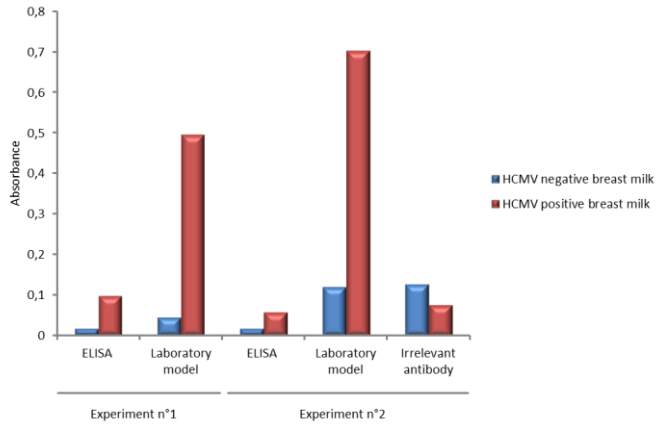
VIRUMILK: Rapid diagnostic test for HCMV detection in breast milk from lactating women of preterm infants less than 33 weeks

S Py[1], B Wacogne[1, 2], L Pazart[1], A Coaquette[3], W Boireau[2], G Thiriez[4] and G Herbein[3, 5]

[1]*INSERM-CIC 1431, Besançon University Hospital, Besançon, France*
[2]*Institute FEMTO-ST, University of Franche-Comté, Besançon, France*
[3]*Laboratory of Virology, Besançon University Hospital, Besançon, France*
[4]*Department of Neonatal Medicine, Besançon University Hospital, Besançon, France*
[5]*UPRES EA4266, SFR FED 4234, Pathogens and Inflammation Laboratory, Department of Virology, University of Franche-Comté, Besançon, France*

Human cytomegalovirus (HCMV) is the leading cause of neonatal viral infection and can have a significant impact on the neurosensory development of newborns and especially preterm infants. HCMV infection may result from maternal-fetal transmission during pregnancy (congenital infection) or postnatal transmission. While congenital HCMV infection affects about 2-5% of very preterm infants, the risk of postnatal infection, particularly through breast milk, is much higher in this population (prevalence of about 20%)[1,2]. Many learned societies wonder about the interest to inactivate HCMV (by freezing or pasteurization) in breast milk in order to reduce or eliminate contamination of these children. However, freezing is relatively inefficient to reduce contamination and pasteurization drastically alters the nutritional quality of the milk[3]. Therefore, a systematic preventive treatment of breast milk for very preterm infants is not currently recommended. An alternative approach could consist in detecting HCMV in breast milk to target at-risk situations. Currently, viral status of breast milk is not explored in practice and, depending on the health centers, breastfeeding is continued as such or milk is systematically inactivated.
To address this problem, and in the current context of breastfeeding promotion, the main objective of VIRUMILK is to develop a simple, rapid and low-cost detection system at patient's bedside, namely a Rapid Diagnostic Test (RDT) based on lateral flow immunochromatography for HCMV detection in the breast milk from lactating mothers in order to prevent the postnatal HCMV infection of preterm newborns less than 33 weeks. The principle of the test is very easy and is based on a sandwich format. The capture antibody is immobilized directly on the migration membrane. A detection antibody conjugated to colloidal gold will be captured at the Test line when HCMV particles are present in the breast milk sample. Signal at the control line, based on the detection on anti-species antibodies will validate the correct functioning of the lateral flow device.
Various interaction experiments have been conducted with "home-made" polyclonal anti-HCMV antibodies, concentrated intracellular HCMV derived from lysed cells and a commercial detection labeled antibody. Results show a capture and/or a detection of HCMV according to the type of experiments. Moreover preliminary positive results were obtained with artificially contaminated breast milk samples in ELISA (Enzyme-Linked Immunosorbent Assays) experiments and with a home-made laboratory device consisting of a fluidic system containing a biochip inserted into a cartridge. Virus concentrations as low as 6 ng/mL can be detected by this simple opto-fluidic device with a higher absorbance value than the one obtained with ELISA technique. This suggests that lower virus concentrations of virus could be detected with a more sophisticated device (see figure below). These preliminary experiments opens the way to the fabrication of bedside RDT.



HCMV detection in artificially contaminated breast milk (~6 ng/mL) by ELISA and with our laboratory device. *Results of two different experiments performed with two distinct batches of capture antibodies are presented. The highest absorbance values are obtained by using HCMV positive breast milk in the laboratory model.*

In conclusion, the blend between medical and engineering research will ensure the translation of VIRUMILK's findings to the clinics with a ready-to-use HCMV RDT to curtail the important disease burden associated with HCMV infection.

[1] Hamprecht K., Maschmann J., Vochem M., et al. "Epidemiology of transmission of cytomegalovirus from mother to preterm infant by breastfeeding". Lancet. 357: pp. 513-518, 2001.
[2] Kurath S., Halwachs-Baumann G., Muller W., et al. "Transmission of cytomegalovirus via breast milk to the prematurely born infant: a systematic review". ClinMicrobiol Infect 16: pp. 1172-1178, 2009.
[3] Buxmann H., Miljak A., Fischer D., et al. "Incidence and clinical outcome of cytomegalovirus transmission via breast milk in preterm infants </= 31 weeks". Acta Paediatr. 98(2): pp. 270–276, 2009.