# Ebola point-of-care diagnostics development for low-resource settings

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In March 2012, West Africa experienced a sudden outbreak of the Ebola virus. It started in Guinea, then spear to Sierra Leone, Liberia and other countries. The most recent outbreak in Liberia was declared in the evening of June 9th, 2016. This virus can cause hemorrhagic fever in human and other mammals and its fatality rate is around 50%. Thus, an optimized detection method that is inexpensive, rapid and easy-to-use is badly needed of and this is the final goal of this project. The method must be stable, highly-efficient, and have extremely high specificity and sensitivity. It must not depend on a well-equipped lab or require intensive labor to produce.

Hyperbranched rolling circle amplification (hRCA) can work in isothermal conditions and give a $10^9$ fold amplification. Combined with a simple and fast detection method like a colorimetric assay this could be a very good method to detect the Ebola virus.

Many reaction conditions have been investigated to optimize the hRCA. The optimal hRCA temperatures for the phi29 DNA polymerase and the Bst 2.0 warmstar polymerase were confirmed. The Bst 2.0 warmstar polymerase showed relatively weak specificity, but was highly efficient in contrast to the phi29 DNA polymerase. We designed three different reaction systems with many corresponding probes and primers in the project. The Original Mouse and the Ebola3small_spc systems can amplify in hRCA, while the Ebola3 small system cannot. Likewise, some probes and primers were effective but some were not. In terms of the experimental design, the whole method contains ligation, followed by amplification and then detection. For using colorimetric assay as a detection method, the agents and the excess padlock probes from the ligation step must not influence amplification. However, experiments demonstrated that some ligation reagents and high concentration excess padlock did affect the amplification.

Many problems were solved in this project, but some new problems also arose. In order to make this inexpensive, rapid and easy-to-use Ebola detection method useful, more experiments are needed to optimize the hRCA and further study of the best colorimetric assay should be performed.