Development of GenomEra-assay for noro-, adeno- and rotaviruses

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BIOTECHNOLOGY (Tech.)

INTRODUCTION

Gastroenteritis is one of the leading causes of morbidity and mortality worldwide. The disease can be caused by a virus, bacterium or parasite, and the infection can be caught from another person or contaminated food or water. As the symptoms resemble each other, it is not possible to differentiate the pathogen causing the disease without a specific and sensitive diagnostic assay. Even though in most cases patient does not need hospitalization, the burden of the disease is still extremely significant.
Prevalence of gastroenteritis annually:

4,5 billion cases &
1,5 million deaths

➔ **Diagnostic need for an effective gastroenteritis assay is undeniable**

Combining the detection of multiple analytes to a single assay offers a rapid and cost-effective solution to meet this demand.

AIM

To develop a GenomEra multianalyte assay to identify three most common viruses causing gastroenteritis.

MATERIALS AND METHODS

GenomEra CDX® is an automated PCR-platform intended for molecular testing of pathogens causing infectious diseases. Utilizing fluorescent labels emitting at different wavelengths, several pathogens can be detected at once. The assay is based on TaqMan chemistry and includes a reverse transcriptase step, as the genetic material of noro- and rotaviruses is RNA.

Suitable PCR primers and probes are the basis of a functional and sensitive assay. We designed several new primers and probes for all of the three analytes based on a broad sequence analysis. The performance of the alternative combinations were tested with the GenomEra device, first in singleplex reactions and later multiplexed.

RESULTS

Sequence design	Singleplex reactions	Multiplex reactions
- Dozens of theoretically working primers and probes were designed *in silico* - most potential ones were selected for *in vitro* screening	- Potential primers and probes were tested one analyte at a time - potential combinations found for all analytes - most potential ones were selected for the next step	- Potential primers and probes were tested in duplex and triplex reactions - potential combinations found for norovirus and adenovirus

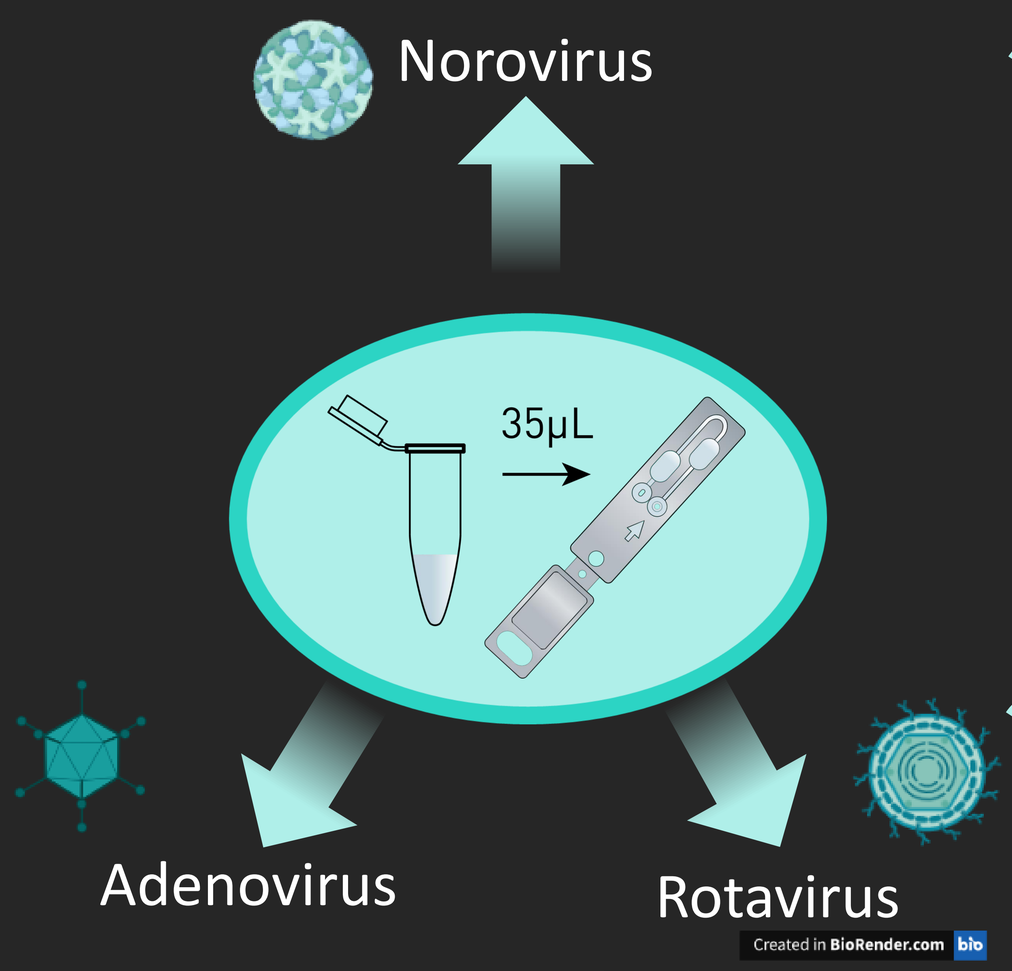

Fig. 1. *Assay is performed on a faecal sample. After short sample processing protocol, it is added to a test chip containing all other necessary reagents for PCR.*

NEXT STEPS

1. Combining rotavirus oligonucleotides to the assay
2. Optimization of concentrations of the oligonucleotides
3. Developing and combining the sample processing control (SPC) into the assay
4. Transfer to production
5. Analytical studies and performance evaluations

➔ Finished product to be CE IVD marked

CONCLUSIONS

Although the development of the assay is still ongoing, the results give a strong indication that the final product is able to effectively detect whether the sample includes any of the three most common viral causes of gastroenteritis. The assay will have diagnostic value and market potential when finished.