ascenion Technology transfer for academic research
A company of the LifeScience Foundation

REFERENCE NUMBER
TO 02-00413

RAPID DETECTION OF SHIGA TOXIN PRODUCING *E. COLI* (STEC)

Keywords: Shiga toxin, STEC, EHEC, *E. coli*, HUS, Diarrhea

INVENTION NOVELTY

This novel assay allows fast functional detection of Shiga toxin-producing *E. coli* (STEC) in culture supernatants, cell suspensions and single cell colonies based on innovative chromogenic enzyme substrates and a FRET readout.

VALUE PROPOSITION

Shiga toxin-producing *E. coli* (STEC) including enterohemorrhagic *E. coli* (EHEC) are a diverse group of bacteria and can cause bloody diarrhea and intestinal inflammation as well as the life-threatening hemolytic uremic syndrome (HUS).
Shiga toxin (Stx), the main virulence factor expressed by the pathogens, is a ribosome-inactivating protein. It inhibits protein synthesis by targeting a special RNA-structure within the ribosomal 60S unit - the so-called Sarcin Ricin Loop (SRL). Stx enzymatically depurinates an adenine, followed by cell death.
Conventional diagnostic methods for STEC are either time- and labor-intensive, detect only a specific subset within the diverse STEC group, or detect the Shiga toxin gene, or Stx but not Stx activity. However, enzymatic activity is the underlying cause for the development of severe symptoms of STEC infection and is found in all STEC.
The inventive method facilitates and accelerates the detection of disease-causing STEC infections substantially. It provides not only a fast point-of-care diagnostic, but offers as well a powerful tool for the prevention of STEC contaminations in food industry.

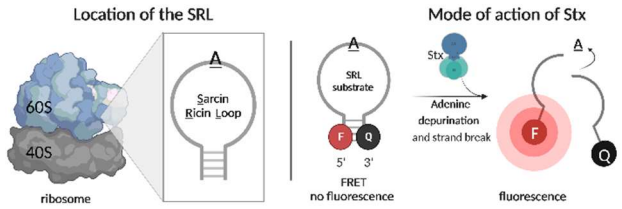

Fig.1: Principle of an enzymatic assay based on Stx N-glycosidase activity
(Source: Ramming et al. 2023,
doi: https://doi.org/10.1101/2023.10.12.562006)

TECHNOLOGY DESCRIPTION

Scientists from RKI and HZI designed StxSense sequences acting as target structures for measuring the glycosidic enzyme activity of Stx. For this purpose, SRL was mimicked using an artificially prepared ssDNA substrate and labeled with a fluorophore (F) and a quencher (Q) at the 5' and 3' ends. In the absence of Stx activity, cleavage of the substrate does not occur, and F and Q remain in spatial proximity, not leading to a fluorescence signal. In the presence of enzymatically active Stx the SRL sequence is depurinated and the substrate is cleaved. As a result, the signal of the fluorophore - no longer blocked by the quencher - can be measured with a fluorescence detection device, e.g. a real-time cycler (Fig.1).

COMMERCIAL OPPORTUNITY

The technology is offered for licensing or co-development.

DEVELOPMENT STATUS

A superior substrate was identified out of several ssDNA substrates based on the SRL sequence of *Rattus norvegicus* and reaction conditions were optimized. Proof-of-concept was achieved with samples from STEC culture supernatants as well as with single cell colonies successfully detecting Stx-subtypes Stx2a-g and Stx1a, Stx1c, and Stx1d independent of the STEC serotype and Stx-containing *Shigella* ssp.. In total, 76 STEC or *Shigella* strains were analysed and Stx production correctly determined. The limit of detection lies in the range of other methods, such as ELISA. No unspecific reaction with Stx-negative gut bacteria were observed.

PATENT SITUATION

A European Patent was filed in May 2023.

HZI HELMHOLTZ Zentrum für Infektionsforschung

Ascenion GmbH
Herzogstraße 64
D-80803 München
info@ascenion.de
www.ascenion.de

Licensing Contact
Dr. Sabina Heim
Senior Technology Manager
T: +49 531 618120-90
heim@ascenion.de