An antibody free targeted LC-MS/MS quantitation method to monitor tetanus toxin (TeNT) during vaccine production

Commonly used acronym: *TeNT quantifcation*

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Contact person

Celine Vanhee

Organisation

Name of the organisation Sciensano

Department Chemical and physical health risks

Specific Research Group or Service Medicines and health care products

Country Belgium

Geographical Area Brussels Region

SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Basic Research, Regulatory use - Routine production, Translational - Applied Research
Type of method	In vitro - Ex vivo

DESCRIPTION

Method keywords

Antibody free protein quantification

targeted LC-MS/MS

tetanus neurotoxin

Cell culture expression

bacteria

Clostridium tetani

Consistency approach

Tetanus vaccine

ICH

VAC2VAC project

Cell culture expression

Scientific area keywords

vaccine production

toxins

3R approach

Method description

The tetanus neurotoxin (TeNT) is one of the most toxic proteins known to man, which prior to the use of the vaccine against the TeNT producing bacteria Clostridium tetani, resulted in a 20% mortality rate upon infection. The clinical detrimental effects of tetanus have decreased immensely since the introduction of global vaccination programs, which depend on sustainable vaccine production. One of the major critical points in the manufacturing of these vaccines is the stable and reproducible production of high levels of toxin by the bacterial seed strains. In order to minimize time loss, the amount of TeNT is often monitored during and at the end of the bacterial culturing. The different methods that are currently available to assess the amount of TeNT in the bacterial medium suffer from variability, lack of sensitivity, and/or require specific antibodies. In accordance with the consistency approach and the three Rs (3Rs), both aiming to reduce the use of animals for testing, inprocess monitoring of TeNT production could benefit from animal and antibody-free analytical tools. We have developed and validated of a new and reliable antibody free targeted LC-MS/MS method that is able to identify and quantify the amount of TeNT present in the bacterial medium during the different production time points up to the harvesting of the TeNT just prior to further upstream purification and detoxification. The quantitation method, validated according to ICH guidelines and by the application of the total error approach, was utilized to assess the amount of TeNT present in the cell culture medium of two

TeNT production batches during different steps in the vaccine production process prior to the generation of the toxoid. The amount of TeNT generated under different physical stress conditions applied during bacterial culture was also monitored. A targeted LC-MS/MS method was developed and validated for the purpose of quantitation of TeNT expressed by Clostridium tetani during the production process to generate the tetanus toxoid vaccine antigen. This method is able to detect TeNT present in the bacterial growth medium with a detection limit of 0.025 Lf/mL and is able to accurately quantify TeNT in the same complex matrix with a LLOQ of 0.25 Lf/mL.

Lab equipment

LC-MS/MS device dedicated for selective reaction monitoring and basic laboratory material (micropipete, centrifuge, speed vac,...).
Reference protein (in case of a protease resistant protein) and stable isotope labeled peptide standards.

Method status

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

Development of a novel and antibody free methodology to quantify the amount of tetanus neurotoxin (TeNT) present in the bacterial medium during vaccine manufacturing.
The method is 10-times more sensitive than the already described LC-MS methodology.
The method has been validated according to ICH guidelines and by the application of the total error approach.
The methodology has proven to be suitable for real-life in-process monitoring of TeNT production.

Challenges

Require specialized machinery and dedicated well trained personnel.

Modifications

Machine (brand) dependent settings.

Future & Other applications

Our methodology can also be used to support the optimization of culture process or to assess the impact of changes in culturing conditions.
This method can serve as an incentive to develop other protein antigen quantification methodologies

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Antoine Francotte, Raphael Esson, Eric Abachin, Melissa Vanhamme, Alexandre Dobly, Bruce Carpick, Sylvie Uhlrich, Jean-François Dierick, Celine Vanhee. Development and validation of a targeted LC-MS/MS quantitation method to monitor cell culture expression of tetanus neurotoxin during vaccine production, Talanta, Volume 236, 2022, 122883, ISSN 0039-9140, https://doi.org/10.1016/j.talanta.2021.122883.

Associated documents

francotte et al 2022.pdf

Links

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