

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

**Commonly used acronym:** TeNT quantification

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### **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**

<b>The Method relates to</b>	Human health
<b>The Method is situated in</b>	Basic Research, Regulatory use - Routine production, Translational - Applied Research
<b>Type of method</b>	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 Uhlich, 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

[paper](#)  
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