

## Report concerning the collaborative study for establish of reference standard efficacy of Teicoplanin

### Raport asupra studiului colaborativ pentru stabilirea potentiei standardului de referință: Teicoplanina

Simona Sturzu, Daniela Tîrșinoagă, Ioana Tihulcă, Mariana Dumitrache,  
Alina Drăghici, Valentina Năstase

Institutul pentru Controlul Produselor Biologice si Medicamentelor de uz Veterinar Bucuresti

Correspondence: [sturzu.simona@icbmv.ro](mailto:sturzu.simona@icbmv.ro)

**Keyword:** *collaborative study, teicoplanin, diffusion method, latin squares*

**Cuvinte cheie:** *studiu colaborativ, teicoplanina, metoda difuzimetrică, pătrate latine*

#### Abstract

The Microbiology Laboratory from the Institute for the Control of Veterinary Biological Products and Medicines participated to a collaborative study in order to determine the potency of teicoplanin CRS, batch 2, according to the protocol sent by the EDQM (European Directorate for the Quality of Medicines) - coordinator of the study. The purpose of the study was to determine the potency of the batch 2 teicoplanin in the characterization of the substance as reference standard. Potency was determined by microbiological method, based on comparing the inhibition zones of growth of micro-organisms sensitive to those of a reference standard, as specified in European Pharmacopoeia, Chapter 2.7.2. - Microbiological testing of antibiotics. After analysis of received data from participants and their statistical processing by the EDQM, the potency of the batch 2, teicoplanin - reference materials, was established in 50150UI/fiola.

#### Rezumat

Laboratorul Control Microbiologic din cadrul Institutului pentru Controlul Produselor Biologice si Medicamentelor de uz Veterinar a participat la studiul colaborativ pentru determinarea potentiei lotului 2 de teicoplanină, conform protocolului trimis de EDQM (Directoratul European pentru Calitatea Medicamentelor), coordonatorul studiului. Scopul studiului a fost de a stabili potentia teicoplaninei, în vederea caracterizării cât mai exacte a lotului 2 de substanță de referință. Potenta a fost determinată prin metoda microbiologică, bazată pe compararea zonelor de inhibare a creșterii unui microorganism sensibil, cu cele ale unui standard de referință, conform prevederilor din Farmacopeea Europeană, editia a 8 a, capitolul 2.7.2. – testarea microbiologică a antibioticelor. În urma analizei si coroborării rezultatelor primite de la participantii si a prelucrării statistice a acestora de către EDQM, valoarea potentiei lotului 2 de teicoplanina, substanta de referință, a fost stabilită la 50150 UI/fiolă.

#### Introduction

The Microbiology Laboratory from the Institute for the Control of Veterinary Biological Products and Medicines participated to a collaborative study in order to determine the potency of teicoplanin CRS, batch 2, according to the protocol sent by the EDQM (European Directorate for the Quality of Medicines) - coordinator of the study.

Participation was due to the inclusion of Microbiology Control Laboratory in this collaborative study, as an independent

verification purposes jurisdiction on analyzes they perform. These studies are important as tools for verification of traceability in quality assurance process, but also as tools for validation of working procedures used under testing laboratories, accredited or undergoing accreditation.

Teicoplanin is a complex glycopeptide antibiotic produced by fermentation of *Actinoplanes teichomyceticus*, and which contain six sub closely related glycopeptide.

Teicoplanin shows in vitro bactericidal activity against gram-positive bacteria and

anaerobic bacteria. Microbiological spectrum of teicoplanin covers *Staphylococcus*, including aureus sensitive or resistant to methicillin, *Staphylococcus pneumoniae*, *Streptococcus* (*Str. pyogenes*, *Streptococcus* from the *Viridans* group) and *Enterococcus faecalis*.

Teicoplanin inhibits the growth of susceptible microorganisms by interfering biosynthesis cell wall at a different level in comparison with beta-lactam antibiotics.

Therapeutic indications of teicoplanin include skin and soft tissue infections, bone and joint infections, pneumonia and respiratory tract infections, bacteremia, septicemia, sepsis, urinary tract infections, infective endocarditis, peritonitis, etc.

## 1. Materials and Methods

Working Samples and Protocol were submitted by EDQM to all participating laboratories. The study enrolled five laboratories from different EU countries, which received numeric codes, from 1 to 5.

Each laboratory involved in the study received:

- 3 vials of teicoplanin powder substance WHO reference standard (approximately 50000 IU / mg), 52 mg / vial
- 6 vials of teicoplanin powder, lot 2, in order to determine the potency (the potency estimated at 51550 IU / vial), 50 mg / vial.

Working method was applied by the Microbiological Control and Efficacy of Biocides Service - Microbiological Control Laboratory, in respect of the conditions imposed by the working protocol of the study.

The principle of the method is based on the dose-response model that is proportional raport between the concentration of antibiotic and the inhibiting growth of the test microorganism.

Potency estimation was conducted on 6 samples by diffusion method, using:

- test microorganism *Bacillus subtilis* ATCC 6633,
- culture medium - medium H, prepared according to the requirements of the European Pharmacopoeia

- pH 6 buffer.

Samples and reference substance stock dilutions were prepared according to the protocol received from the EDQM.

In order to test the validity of three different doses were used for both the test sample and the reference material used, that dilutions of 1/160, 1/80 and 1/40 (rate of dilution 2).

Plates were prepared with the media and test microorganisms needed for each sample separately.

After solidification of culture medium, on the surface of the medium from the plates were placed 4 metallic cylinders using sterile forceps.

After preparing the working dilutions, there were dispensed 0.4 ml of standard and sample in the corresponding cylinders.

The plates prepared were left for 1-4 hours at room temperature (pre-incubation period), after which the plates were incubated for 18 hours at 35-37°C.

Special attention was paid to handle and transfer of the plates from the working desk in the thermostat.

The diameters of the zones of inhibition were measured visually using digital micrometer. Sample concentration was calculated using an Excel sheet.

## 2. Results and discussion

Reporting results was made by e-mail, as specified in the test protocol, using an Excel file for each individual test. The calculation method used was Latin squares.

The results obtained and sent the Microbiological Control Laboratory for all 6 samples, are presented below (Table 1).

Deviations of the results obtained for all the 6 samples, reported on the potency determined by EDQM, were:

- Sample no. 1 to 5.8%
- Sample no. 2 to 5.9%
- Sample no. 3 to 7.9%
- Sample no. 4 - 7.6%,
- Sample no. 5 to 9.8%
- Sample no. 6 to 7.5%.

Table 1

Results obtained by the Microbiological Control Laboratory comparing with EDQM results

Crt. No	Results calculated by the Microbiological Control Laboratory (IU / vial )		Results calculated by EDQM (IU / vial )	
	Estimated Potency	Confidence limits 95 %	Estimated Potency	Confidence limits 95 %
1	48598	94,5 - 105,8 %	48627	94,5 - 105,8%
2	50783	94,4 - 105,9 %	50812	94,4 - 105,9%
3	50696	92,9 - 107,6 %	50726	92,9 - 107,6%
4	50000	92,7 - 107,9 %	50029	92,7 - 107,9%
5	49501	91,0 - 109,8 %	49530	91,0 - 109,8%
6	50099	93,0 - 107,5 %	50128	93,0 - 107,5%

The combined result from all the 6 samples was 49 929 IU / vial.

All 6 results sent by the Microbiological Control Laboratory were accepted as valid and were statistically processed. For the other six results, accepted as homogenous, the average potency was 49,929 IU / vial (95% confidence limits, values of 97.4 - 102.7%).

The results of participating laboratories were compared with those obtained in the EDQM laboratory.

EDQM report setting out the results of participants, comments relating to the confidence interval of the study, the potency value, deviation from linearity, homogeneity readings, the average of the results, standard deviation, relative standard deviation, etc was sent to each participating laboratory. Evaluation of the results was done using statistical calculation model -parallel lines- using SAS System program and CombiStats.

### 3. Conclusions

After analyzing and corroborating the results received from the participants and statistical processing of them by EDQM, Lot 2 teicoplanin potency value - reference substance, was set at 50,150 IU / vial.

### Biblografie

1. European Pharmacopoeia 8th edition: Chapter 2.7.2 . Biological tests - microbiological testing of antibiotics
2. European Directorate for Quality of Medicines (EDQM), working protocol for determining the potency teicoplanin CRS , Lot 2 .
3. European Directorate for Quality of Medicines ( EDQM ) , the report to determine the potency teicoplanin CRS , Lot 2