Vol. 7 (1). May - June 2013

Report concerning results of proficiency testing laboratory on assay of tobramycine and nystatin by microbiological method

Raport privind rezultatele testului de competenta a laboratoarelor pentru determinarea nistatinei si tobramicinei prin metoda microbiologica

Simona Sturzu, Daniela Tirsinoaga, Ioana Tihulca, Alina Karina Draghici
Institute for Control of Veterinary Biological Products and Medicines, Bucharest

Keywords: *proficiency testing schemes (PTS), Inter-Laboratory Comparisons, tobramycin, nystatin*
Cuvinte cheie: *shema de competenta a laboratoarelor, comparari interlaboratoare, tobramicina, nistatina*

Abstract

The present study describes the results obtained by the Microbiological Control Laboratory from Institute for Control of Biological Products and Veterinary Medicines after participating in the proficiency testing scheme study on microbiological assay of nystatin and tobramycin. The proficiency testing scheme was organized by European Directorate for the Quality of Medicines and Health Care. The microbiological method consisted of a cylinder-plate agar diffusion assay using *Bacillus subtilis* ATCC 6633 for tobramycin and *Saccharomyces cerevisiae* for nystatin as the test microorganism. The means of results were 108.70 % of label claim for nystatin and 104.70% of label claim for tobramicin. The Z scores were 0.14 for tobramycin and 1.40 for nystatin, the assigned value used for booth samples was 105.4% for tobramycin and 101.7% for nystatin. The performance of Microbiological Control Laboratory was very good for both samples.

Rezumat

Lucrarea descrie rezultatele obtinute de catre Laboratorul Control Microbiologic din cadrul Institutului pentru Controlul Produselor Biologice si Medicamentelor de uz Veterinar dupa participarea la shema de competenta a laboratoarelor pentru determinarea nistatinei si tobramicinei prin metoda microbiologica (PTS 130). Schema de competenta a laboratoarelor a fost organizata de catre Directoratul European pentru Calitatea Medicamentelor. Metoda microbiologica utilizata se bazeaza pe difuzia in agar a substantei de analizat (repartizata in cilindri), folosind ca microorganisme test *Bacillus subtilis* ATCC 6633 pentru tobramicina si *Saccharomyces cerevisiae* pentru nistatin. Media rezultatelor obtinute a fost de 108,70% din cantitatea inscrisa pe eticheta produsului pentru nistatina si 104,70% din cantitatea inscrisa pe eticheta produsului pentru tobramicin. Scorul Z a fost 0,14 pentru tobramicina si 1,40 pentru nistatina, iar valoarea atribuita de catre EDQM pentru tobramicina a fost 105,4 % si 101,7% pentru nistatin. Performanta Laboratorului Control Microbiologic a fost foarte buna pentru ambele probe.

Introduction

Participation to proficiency tests (laboratory evaluating inter-laboratory tests) is considered mandatory for laboratories accredited according to ISO / IEC 17025.

Checking analyses results with those of other laboratories is one of the most important quality control elements.

Confidence that a laboratory consistently produces reliable results is of major importance to the laboratory itself and the organization it belongs to.

The Microbiological control laboratory from ICBMV is regularly taking part in Proficiency testing schemes organized by EDQM. For PTS 130, 22 laboratories participated that needed to determine the percentage content of tobramycin injection and nystatin suspension from samples labeled A and B, according to European Pharmacopoeia, 2.7.2, The diffusion method.

1. Materials and Methods

The principle of the method has, as a basis, a dose - response model in which the antibiotic concentration is

proportional to the inhibition zone of microorganism growth.

Sample A: tobramicine injection (25 mg/2,5 ml), reference substance, water R - solvent used in preparing the stock, buffer solution pH 8.0, microorganism test - *Bacillus subtilis* ATCC 6633, nutrient agar.

Sample B: nystatin oral suspension (100000 IU/ml), reference substance, dimetihylformamide, potassium dihydrogen orthophosphate solvents used in preparing the stock, microorganism test – *Saccharomices cerevisiae*, nutrient agar.

The plates were prepared with media and microorganisms test needed for each samples.

After medium solidification, 4 metal cylinders were placed on the plate's surface using sterile pens. The stock and working samples as well as the reference substance dilutions were prepared according to EDQM protocol. To assess the validity of the assay 3 different doses (tobramycin: 12 IU/ml, 6 IU/ml, 3 IU/ml and nystatin: 100 IU/ml, 50 IU/ml, 25 IU/ml) of the reference material were used together with an equal number of doses of the test substance having the same presumed activity as the solutions of the reference material. After preparing the working dilution, 0.4 ml of the standard and test sample solution were poured in their corresponding cylinders. The plates were left for 1 – 4 hrs at room temperature as a period of pre-incubation diffusion. The plates were incubated for about 18 hrs at 35 to 37˚C. Care was taken while transferring the plates from laminar bench to incubator.

After incubation, the diameter of the zone of inhibition was measured using a micrometer.

The potency of the sample was calculated and the results were reported as percentage of the label claim in Excel data sheet. The precision of the assay was such that the fiducially limits of error were not less than 95% and not more than 105% of the estimated potency.

2. Results and Discussion

In the present studies, Microbiological assay estimated the quantity of tobramycin and nystatin present in the sample A and B.

Table 1 shows the content of Tobramycin in sample A, for 3 independent determinations.

Determination	SAMPLE A (Tobramycin injection) % of label claim
1	105,10
2	103,80
3	105,20
Mean	104,70
SD	0,7810
RSD	0,7517

The content was calculated taking into account that each 1000 IU is found to be equivalent to 1 mg of tobramycine.

The mean of Potency was 104.70%, standard deviation was 0.7810 and relative standard deviation was 0.7517.

Table 1 shows the content of Nistatin in sample B, for 3 independent determinations.

Determination	SAMPLE B (Nystatin oral suspension) % of label claim
1	109,91
2	107,25
3	108,96
Mean	108,70
SD	1,3479
RSD	1,0439

The content was calculated taking into account that each 5825 IU is found to be equivalent to 1 mg of nystatin.

The mean of Potency was 108.70%, standard deviation was 1.3479 and relative standard deviation was 1.0439.

The results of the studies were sent to EDQM for examination. After the examination of the data received from laboratories involved in this study, EDQM has calculated, for each sample, the mean value, the standard and relative deviation and the Z-score.

For consistency, data are commonly reported using a *cut-off value*, often < -2 and > +2 Z-scores.

The rationale for this is the statistical definition of the central 95% of a distribution as the *"normal"* range, which is not necessarily based on the optimal point for predicting functional outcomes.

For sample A, the mean of all the results (from 22 laboratories) was 105.7 % with a standard deviation of 5.28% and for sample B the mean of all the results (from 22 laboratories) was 96.5% with a standard deviation of 23.80%, according to EDQM.

For the Microbiological Control Laboratory the Z- score was – 0.14 for tobramycin and + 1.40 for nystatin. The assigned value used for samples was 105,4 % for tobramycin and 101,7 % for nystatin.

3. Conclusions

1. The results obtained by the Microbiological Control Laboratory has found that the Z scores are in the established interval (-0.14 for tobramycin and +1.40 for nystatin).
2. The performance of the Microbiological Control Laboratory was very good for both samples and EDQM sent the *"Attestation of participation in proficiency testing scheme"* in 13/09/2012.

References

1. **European Pharmacopoeia**, 7 edition, chapter 2.7.2
2. **EDQM Working Protocol** - for microbiological assay of nystatin and tobramycin (PTS 130)
3. **EDQM Report -** concerning microbiological assay of nystatin and tobramycin (PTS 130)