Ljilja D. TOROVIĆ \textsuperscript{1,2}\*  

\textsuperscript{1} University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Hajduk Veljkova 3, Novi Sad 21000, Republic of Serbia \textsuperscript{2} Institute of Public Health of Vojvodina, Futoška 121, Novi Sad 21000, Republic of Serbia

LABORATORY COMPETENCE EVALUATION THROUGH PROFICIENCY TESTING – MYCOTOXINS IN FOOD

ABSTRACT: Laboratory for analysis of mycotoxins in food at the Institute of Public Health of Vojvodina (Novi Sad, Serbia) participated in 15 proficiency testing schemes in period 2012–2016, comprising 22 determinations of regulated mycotoxins: aflatoxins B1, B2, G1, G2 and M1, ochratoxin A, deoxynivalenone, zearalenone, fumonisins and patulin, in different food commodities: wheat, corn, barley, breakfast cereals, infant food, milk, wine and fruit juice. Analyses were carried out by high performance liquid chromatography with ultraviolet (patulin, deoxynivalenol) or fluorescence detection (aflatoxin M1, ochratoxin A, zearalenone) using o-phthalaldehyde precolumn derivatization (fumonisins) or UV postcolumn derivatization (aflatoxins B1, B2, G1, G2), following clean-up on immunoaffinity columns with specific antibodies, except in case of patulin when solvent extraction and solid-phase C-18 clean-up were used. Laboratory performance assessed in terms of $z$ scores showed all satisfactory results. In depth evaluation revealed following distribution of $z$ scores (absolute values): 59.1\% up to 0.5, 36.4\% between 0.5 and 1.0, and 4.5\% above 1.0. Analysis of trends performed for multiple determinations of individual mycotoxins showed several changes of $z$ score to better or worse rank. Overall assessment of the performance in proficiency testing demonstrated laboratory competence for analysis of mycotoxins in food.

KEYWORDS: quality assurance, proficiency testing, food, mycotoxins

INTRODUCTION

Participation in proficiency testing (PT) is a powerful element of quality assurance plan for the analytical laboratories, required for the ones seeking recognition of competence through accreditation against the standard ISO/IEC 17025 (ISO, 2005). A laboratory needs to establish an appropriate PT strategy considering relevance of PT schemes and frequency of participation. To achieve

\* Corresponding author. E-mail: ljilja.torovic@mf.uns.ac.rs
that, a laboratory has to collect comprehensive information on the availability and scope of PT schemes in the areas of its work. A number of key principles need to be considered: PT scheme should resemble the laboratory’s routine samples, analytes and concentration levels; PT items should be treated as routine samples; evaluation of the results should take into account the measurement uncertainty; unsatisfactory or repeated questionable results must be subjected to thorough root cause investigation followed by corrective actions; analysis of trends over several PT rounds should be performed and interpreted in order to improve the performance (Eurachem, 2011). PT schemes have to be organized according to principles defined in standard ISO/IEC 17043 (ISO, 2010). A laboratory should understand the basic statistics and performance scoring used by the PT providers.

In a laboratory monitoring chemical food safety, analyses need to be carried out to investigate occurrence of numerous chemicals in wide variety of food commodities. Implementation of public health protection policies in many countries led to regulation of the maximum level of chemicals in food with potential adverse health effects. Serbian legislation regarding this issue (Ministry of agriculture, 2014) is harmonized with the European Union regulation (European Commission, 2006). Selection of contaminants, based on the severity of potential health effects and the extent of exposure through food, included several mycotoxins: patulin (PAT), aflatoxins (AFs), ochratoxin A (OTA), zearalenone (ZEA), deoxynivalenol (DON) and fumonisins (FUM). Mycotoxins are naturally occurring toxic substances, secondary metabolites of filamentous fungi.

Patulin production is connected to the fungi belonging to *Penicillium, Aspergillus* and *Bysochlamys* species, growing on fruits, especially apple. Apple juice is considered a major source of patulin in human diet. Toxicological profile of patulin could be briefly summarized by provisional maximum tolerable daily intake (PMTDI) of 0.4 µg/kg bw/day (JECFA, 1995) and International Association for Research on Cancer (IARC) classification in group 3 (not classifiable as to its carcinogenicity to humans) (IARC, 1986).

Aflatoxins are secondary metabolites of the fungi *Aspergillus flavus* and *A. parasiticus*, and less frequently other *Aspergillus* species. AFs are prevalent in food crops, particularly corn, peanuts (groundnuts), oilseeds and tree nuts. AFM1 is hydroxylated metabolite of AFB1, excreted in milk. AFB1, the most toxic aflatoxin, is extremely potent carcinogen (IARC group 1) (IARC, 2012) and therefore a health based guidance value has not been established. AFM1 is classified in IARC group 2B (IARC, 1993).

Ochratoxin A is produced by the fungi representing *Aspergillus* and *Penicillium* genera. It is most often found in cereals, grape and wine. OTA exhibits renal toxicity. Tolerable weekly intake is 120 ng/kg bw (European Food Safety Authority, 2006); IARC group 2B (potential carcinogen to humans) (IARC, 1993).

Zearalenone is biosynthesized by a large range of *Fusarium* fungi on cereal crops, especially corn. The critical effects result from its estrogenic activity leading to hyperestrogenism. Tolerable daily intake is 0.25 µg/kg bw (European Food Safety Authority, 2011); IARC group 3 (IARC, 1993).
Deoxynivalenol is type B trichotecene primarily associated with *Fusarium graminearum* fungi. It co-occurs in cereal-based food together with its acetylated derivates. The primary toxic effect is inhibition of protein synthesis. PMTDI 1 µg/kg bw DON and its acetylated derivatives (3-Ac-DON and 15-Ac-DON) (JECFA, 2011); IARC group 3 (IARC, 1993).

Fumonisins are a group of structurally related mycotoxins primarily produced by *Fusarium verticillioides* and *Fusarium proliferatum*. Fumonisin B1 (FB1) and B2 (FB2) are the most abundant and often found as contaminants in corn products. FB1 is the most toxic fumonisin, related to an inhibition of sphingolipid synthesis and increased risk of esophageal cancer in humans. PMTDI 2 µg/kg bw (FB1, FB2 and FB3, independently or combined) (JECFA, 2002); IARC group 2B (IARC, 2002).

The reliability of routine analyses of chemical contaminants in food, reflected in laboratory PT performance, is of paramount importance for food safety evaluation, as well as for health risk characterization taking into account that population exposure assessments are based on the contaminants occurrence data. With regard to that, this report presents technical performance of the Institute of Public Health of Vojvodina – Laboratory for analysis of organic contaminants, in PT schemes for analysis of mycotoxins in food.

**MATERIAL AND METHODS**

Standard solutions and reagents: Standard solutions of PAT and AFM1 were obtained from Supelco (Bellefonte, PA, USA), whereas standard solutions of AFs, OTA, ZEA, DON and FUM were from LCG Standards (Wesel, Germany). Solvents (ethylacetate, hexane, acetonitrile, methanol) were purchased from LGC Promochem (Wesel, Germany); acetic and hydrochloric acid from Carl Roth (Karlsruhe, Germany). Ultrapure water was produced by GenPure Water Purification System (Thermo Scientific, Thermoelectron LED, Langenselbold, Germany).

Samples: The Laboratory ordered 15 PT samples over four years (2012-2016). The PT providers, Fera Science (FAPAS – Food Analysis Performance Assessment Scheme, UK) and Romer Labs (Austria), distributed homogeneous and stable PT samples for analysis (details provided in Table 1 in Results and discussion session).

Sample preparation: Analyses were carried out by single analyte methods. Each mycotoxin was extracted from the food matrix and purified using immunoadfinity columns with specific antibodies (LCTech, Dorfen, Germany / Vicam, Waters, US) following producers’ instructions. The exception was PAT, for which solvent extraction and solid-phase C-18 (Supelco, Bellefonte, PA, US) clean-up were used, according to Arranz *et al.* (2005).

**HPLC analysis:** Analyses were performed on Agilent Series 1100 HPLC system (Agilent Technologies, Wilmington, DE, USA) consisting of degasser (G1322A), quaternary pump (G1311A), autosampler (G1329A), thermostated column compartment (G1316A), UV detector (G1314A), fluorescence detector
(G1321A), and a photochemical post column derivatizer UV 254 (LC-Tech, Dorfen, Germany). Separations were achieved on a reverse phase analytical columns Zorbax SB-C18 (5 µm, 4.6x250 mm; Agilent Technologies, USA) (PAT, AFM1, OTA, DON) and Eclipse XDB-C18 (5 µm, 4.6x150 mm; Agilent Technologies, USA) (AFs, ZEA, FUM). Mobile phases were as follows: water:acetonitrile:perchloric acid (99:4:0.1, 1 ml/min) (PAT); water:methanol: acetonitrile (6:3:1.5, 1.2 ml/min) (AFs); water:acetonitrile (3:1, gradient A:B 25%→70%, 1ml/min) (AFM1); acetonitrile:2%aq.acetic acid (3:2, 1 ml/min) (OTA); acetonitrile:water (53:47, 1 ml/min) (ZEA); methanol:water:acetic acid (A 15:85:0.1, B 1:1:0, gradient A:B 100%→0%, 1ml/min) (DON); water:acetonitrile:acetic acid (A 30:69:1, B 60:39:1, gradient A:B 3:2→0:1, 1ml/min) (FUM). Mycotoxins were detected by ultraviolet (PAT 276nm, DON 220nm) or fluorescence detector (AFM1 λexc 365nm, λem 435nm; OTA λexc 333nm, λem 448nm) using o-phthalaldehyde precolumn derivatization (FB1 and FB2 λexc 335nm, λem 440nm) or UV postcolumn derivatization (AFs λexc 365nm, λem 430nm). According to the scheme propositions, all analytical results were corrected for recovery as determined by the Laboratory in method validation studies.

Laboratory performance was assessed in terms of z scores, as given by the proficiency test provider. The scores were interpreted in the following way: |z|≤2 “satisfactory”, 2<|z|<3 “questionable”, |z|>3 “unsatisfactory”.

RESULTS AND DISCUSSION

Food commodities and mycotoxins analyzed as proficiency test samples are presented in Table 1, as well as PT providers and test codes. Performance of the Laboratory in proficiency testing is presented in Table 2, including data on mycotoxin level assigned by the PT provider, Laboratory result and obtained z scores.

Table 1. Proficiency tests – mycotoxins in food, performed over 2012–2016

<table>
<thead>
<tr>
<th>PT title (PT sample)</th>
<th>Reference (Provider, year/test code)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patulin in apple juice</td>
<td>FAPAS, 2012/1647; 2016/1656</td>
</tr>
<tr>
<td>Mycotoxin contamination in infant food</td>
<td>FAPAS, 2012/04199</td>
</tr>
<tr>
<td>Aflatoxins in corn</td>
<td>Romer Labs, 2015/M15421A; 2016/ M16411A</td>
</tr>
<tr>
<td>Aflatoxin M1 in milk</td>
<td>FAPAS, 2013/04224</td>
</tr>
<tr>
<td>Ochratoxin A in wine</td>
<td>FAPAS, 2013/17117</td>
</tr>
<tr>
<td>Ochratoxin A in barley flour</td>
<td>FAPAS, 2016/17163</td>
</tr>
<tr>
<td>Deoxynivalenol in breakfast cereals</td>
<td>FAPAS, 2014/22107</td>
</tr>
<tr>
<td>Deoxynivalenol in wheat</td>
<td>Romer Labs, 2014/ M14161D</td>
</tr>
<tr>
<td>Deoxynivalenol in corn</td>
<td>Romer Labs, 2016/16161D</td>
</tr>
<tr>
<td>Zearalenone in breakfast cereals</td>
<td>FAPAS, 2014/22106</td>
</tr>
<tr>
<td>Zearalenone in wheat</td>
<td>Romer labs, 2014/ M14421Z</td>
</tr>
<tr>
<td>Fumonisins in maize flour</td>
<td>FAPAS, 2013/2297; 2016/22133</td>
</tr>
</tbody>
</table>
Laboratory performance assessed in terms of $z$ scores showed all satisfactory results. In depth evaluation revealed following distribution of $z$ scores (absolute values): 13 (59.1%) up to 0.5, 8 (36.4%) between 0.5 and 1.0, 1 (4.5%) above 1.0. Analysis of trends performed for multiple determinations of individual mycotoxins showed several changes of $z$ score to better or worse rank: PAT improved, AFB1 and DON improved and worsened, FUM slightly worsened. PAT and FUM were analyzed in the same type of food commodity, as well as AFs in last two PT rounds, while in case of other mycotoxins it should be noticed that different food commodities could have substantial influence on laboratory performance. Longer time periods and substantial financial resources are needed to obtain enough data to enable analysis of trends over several PT rounds. However, the Laboratory successfully fulfilled the requirements for the four year accreditation cycle according to the rules of Accreditation body of Serbia (2014). Analytical methods for determination of all legally regulated mycotoxins in selected food commodities are in the scope of accreditation of the Laboratory.

Table 2. Performance of the Laboratory in proficiency testing – mycotoxins in food

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Food commodities and results</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PAT</td>
<td>Apple juice Apple juice</td>
<td>Assigned / Lab$^a$ 39.3 / 46.2 45.7 / 48.8</td>
<td>$z$ score 0.8 0.3</td>
</tr>
<tr>
<td>AFS</td>
<td>Infant food Corn$^b$ Corn$^b$</td>
<td>Assigned / Lab$^a$ 0.12 / 0.10 n.p./&lt;LOD 6.0 / 6.2 0.37 / 0.4 6.58 / 5.27 0.72 / 0.64</td>
<td>$z$ score -0.7 0.1 0.3 -0.9 -0.5</td>
</tr>
<tr>
<td>AFM1</td>
<td>Milk powder</td>
<td>Assigned / Lab$^a$ 0.181 / 0.172</td>
<td>$z$ score -0.2</td>
</tr>
<tr>
<td>OTA</td>
<td>Infant food Wine Barley flour</td>
<td>Assigned / Lab$^a$ 0.39 / 0.40 1.94 / 2.3 3.02 / 2.95</td>
<td>$z$ score 0.1 0.8 -0.1</td>
</tr>
<tr>
<td>ZEA</td>
<td>Breakfast cereals Wheat</td>
<td>Assigned / Lab$^a$ 99.6 / 88.8 119 / 125.8</td>
<td>$z$ score -0.5 0.3</td>
</tr>
<tr>
<td>DON</td>
<td>Breakfast cereals Wheat Corn</td>
<td>Assigned / Lab$^a$ 764 / 934 915 / 955 824.73 / 907</td>
<td>$z$ score 1.3 0.3 0.6</td>
</tr>
<tr>
<td>FUM</td>
<td>Maize flour Maize flour</td>
<td>Assigned / Lab$^a$ 765 / 618 350 / 359 1123 / 977 731 / 826 144 / 132 874 / 958</td>
<td>$z$ score -0.8 0.1 -0.5 0.8 -0.4 0.6</td>
</tr>
</tbody>
</table>

$^a$ results in µg/kg.  
$^b$ AFG1 and AFG2 were not present in test material and not found by laboratory.
One of the main objectives of a PT scheme is to help the laboratory to assess the accuracy of its measurements. With regard to this criterion, performance of the Laboratory was also fit for purpose: PAT 107–118%, AFB1 80–103%, AFB2 89–108%, AFM1 95%, OTA 98–119%, ZEA 89–106%, DON 104–122%, FB1 81–113%, FB2 92–103%, FB1+FB2 87–110%.

**CONCLUSION**

Participation in PT plays a highly valuable role providing an objective evidence of the competence of a laboratory. It is worth noticing that, apart from analytical laboratory, customers of laboratory services, accreditation bodies and regulatory authorities also have an interest in PT schemes as a means to independently monitor the validity of measurements.

**REFERENCES**

Accreditation body of Serbia (22 8 2014). Pravila o učešću u međulaboratorijskim poređenjima i programima za ispitivanje osposobljenosti. Date of access 7. 3. 2017. Available at: www.ats.rs.


ОЦЕНА КОМПЕТЕНТНОСТИ ЛАБОРАТОРИЈЕ КРОЗ ТЕСТИРАЊЕ ОСПОСОБЉЕНОСТИ – МИКОТОКСИНИ У ХРАНИ

Љиља Д. ТОРОВИЋ1,2
1 Универзитет у Новом Саду, Медицински факултет, Департман за фармацију, Хајдук Вељкова 3, Нови Сад 21000, Република Србија
2 Институт за јавно здравље Војводине, Футошка 121, Нови Сад 21000, Република Србија

РЕЗИМЕ: Лабораторија за анализу микотоксина у храни Института за јавно здравље Војводине (Нови Сад, Србија) учествовала је у 15 шема за тестирање оспособљености у периоду од 2012. до 2016. године, које су обухватиле 22 одређивања законом регулисаних микотоксина: афлатоксини Б1, Б2, Г1, Г2 и М1, охратоксин А, деоксиниваленол, зеараленон, фумонизини и патулин, у различитим намирницама: пшеница, кукуруз, житарице за доручак, храна за одојчад, млеко, вино и воћни сок. Анализа су урађене методом течне хроматографије високих перформанси с ултраљубичастом (патулин, деоксиниваленол) или флуоресцентном детекцијом (афлатоксин М1, охратоксин А, зеараленон) коришћењем преколонске дериватизације с о-фталалдехидом (фумонизини) или ултраљубичасте постколонске дериватизације (афлатоксини Б1, Б2, Г1, Г2), након пречишћавања применом имуноафинитетне хроматографије с специфичним антителима, изузев у случају патулине, за чије одређивање је примењена екстракција растваражем и пречишћавање на чврстој фази (С18). Учинак лабораторије оценио је на основу постигнутих z скора, при чему су сви резултати били задовољавајући. Детаљна анализа пока зала је следећу расподелу z скора: 59,1% до вредности од 0,5; 36,4% између 0,5 и 1,0; 4,5% изнад 1,0 (као апсолутне вредности). За вишеструка одређивања појединачних микотоксина анализиран је тренд, при чему је уочено неколико промена ранка z скора ка бољем или лошијем. Учешћем у тестирању оспособљености лабораторија је потврдила компетентност за анализу микотоксина у храни.

КЉУЧНЕ РЕЧИ: осигурање квалитета, тестирање оспособљености, храна, микотоксини