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## Analytical Service.

**Romer Labs®** leads the industry in providing quantitative mycotoxin testing service. Our testing services encompass over 20 mycotoxins and employ the latest technology available (HPLC, LC-MS, GC-MS). For over 20 years, Romer Labs® has consistently provided fast, accurate, reliable results for our customers.

**We provide analytical service for mycotoxins in our laboratories in the US, Singapore and Austria.**

Our strong commitment to quality assures our customers that they are receiving the most accurate results in mycotoxin analysis. **Romer Labs®** testing services do not stop at accurate mycotoxin results. Our technical support team has the expertise to interpret the analytical results and our experienced consulting service offers potential solutions to help clients managing their mycotoxin problems. If accurate mycotoxin results and information are essential to your business, Romer Labs® can be of service to you.

## > LITERATURE


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## > IMPRESSUM


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# Spotlights

Volume 1

## > EDITORIAL

*Mycotoxins are undesired contaminants of increasing concern occurring in food- and feedstuffs. Produced by many different species of moulds under various conditions it is almost impossible to avoid their occurrence. They are invisible, odourless and tasteless toxins with a major impact on human and animal health. To make the situation even more complex contaminations with mycotoxins can usually not been correlated with visible mould growth.*
*Mycotoxin related effects range from general immunosuppression in the case of trichothecenes to liver damage and carcinogenicity in the case of aflatoxins. Especially for aflatoxins most countries do already have strict regulation to keep risks at a minimum level. But new regulations are enacted for other toxins as well. E.g. the European commission issued levels for ochratoxin A in various commodities.*
*To avoid contamination of foods we must be able to detect mycotoxins wherever they occur and understand the limitations and benefits of various analytical procedures and detection systems available. Everyone establishing a testing program desires to avoid invalid results as they are often more detrimental than no results. Are you aware of the fact that proper result validation makes the difference?*
*Have a look!*

***Gustav Kichler***

**Feed and feed ingredients can be analysed for mycotoxin-contaminations in numerous ways. Unfortunately the variability in results between different analytical laboratories is high. This fact cannot only be attributed to the crucial sampling procedure, but also to the quality of the methods applied by individual laboratories. For the costumer it is often hard to assess the quality and reliability of analytical results. However, looking at the efforts a lab makes in terms of quality assurance might help to evaluate reports of analytical results. Different measures taken by qualified laboratories are described in the following.**

# Quality control in Mycotoxin Analyses. An option or an absolute must?

by Elisabeth Fuchs

Mycotoxins are secondary metabolites of fungi that occur in almost all agricultural commodities. They are produced by a great variety of fungi in different climates. However, fungal infection does not automatically indicate the presence of mycotoxins. Mycotoxins differ a lot in their chemical structure and physical properties. These differences lead to a great variety of possible effects on human or animal health and have an influence on the analytical method used for detection. The biggest challenge in analysing mycotoxins is usually not the analytical procedure done in the lab but the previous sampling step.

Mycotoxins occur unevenly distributed in so called "hot spots" in which very high concentrations may be present while the largest part of a lot may be uncontaminated. Thus, homogenising and sampling are crucial issues to get a reliable result. When it comes to quality control of feedstuffs, this step is usually done by the farmer himself and he has to be aware of this challenge and his responsibility in this process.

## Tools assuring the quality of a lab

In the lab many other possible sources of mistakes and errors have to be considered and avoided. The procedure beginning with grinding, sub-sampling, extraction, clean up, detection and final evaluation is called the analytical method. The way to a fully validated reliable analytical method is best done in three steps shown in figure 1.

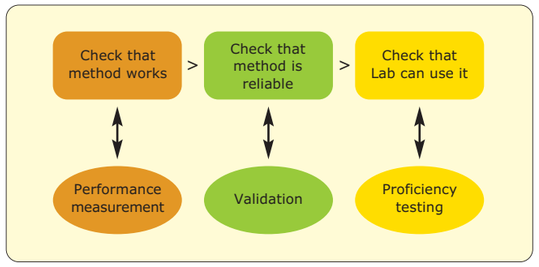


*Fig 1: Steps necessary for a good analytical method [1].*

Analytical methods have to be checked in various ways [3, 4]: After a method is fully developed and the performance of this method is checked it may then be integrated in the routine procedure of the lab. A very important step on the way to a routine procedure is the in house validation. This is usually done with artificially contaminated samples containing different concentrations of a certain toxin. All samples are analysed by means of the new analytical procedure on different days by different operators, even on different instruments. Thus, all possible influencing factors are already involved in the validation procedure.

**The evaluation of the gathered data gives answers to the following questions:**

- **What is the linear range?**
- **What is the detection limit?**
- **What is the limit of quantification?**
- **How precise is the result I get?**
- **How accurate is the result I get?**
- **How robust is the method performed by different operators?**

Based on the data gained in this validation study a system is established to perform routine day to day quality control of the respective method. The tool used for this purpose is a control chart. To establish this chart at least twenty replicates of a homogenous naturally contaminated sample are analysed on different days by different operators. The results are used to build the control chart (figure 2): The red line in the centre indicates the mean of twenty samples. Warning lines are drawn at +/- two times the standard deviation of the mean and control lines at +/- three times the standard deviation of the mean. Whenever samples are analysed routinely with the new method a quality control sample is analysed, too, and its result is entered in the control chart. If this result lies between the warning lines the results of the samples are acceptable and may be reported to the costumer. If this is not the case the method and all possible sources of errors have to be checked until the quality control is acceptable again.

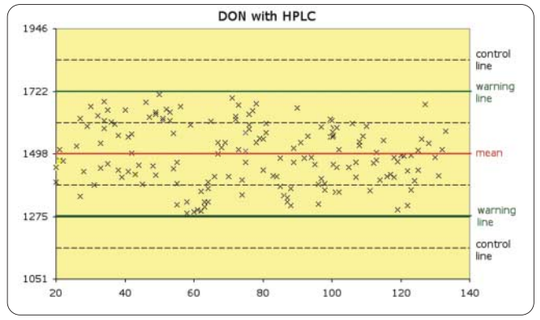


*Fig 2: Example for a Quality Control Chart for assessing the performance of a method under routine conditions.*

## International comparison studies

Besides having an internal benchmark for the quality of a lab it is also necessary to compare the standard to other labs, i.e. to have an external benchmark. It is a well-known fact that although the in house validations of different labs show very good data, they will still report different results of one and the same analysed sample. It could be shown in a number of inter-laboratory comparison studies that the observed variations are mainly due to differences of the calibrant used. Josephs et al. (2000) and Krska et al. (2000) reported coefficients of variation (CV) between the laboratories of more than 40% when the laboratories used their own calibrant [2, 5]. If the calibrant was standardised across laboratories, CVs of 15-28% were achieved in the study. This shows the importance of having a trusted source of mycotoxin standards.

## Proficiency testing

Besides inter-laboratory comparison studies there are so called proficiency testing systems available where every lab can participate. One of the most popular systems in the field of mycotoxins was established by the Central Science Laboratory (CSL, York, U.K.) [6].





**FAPAS® Chemical Analysis of food enables the participating labs to:**

- **demonstrate commitment to high quality standards by providing customers, regulators and accreditation bodies with independent assessment criteria.**
- **demonstrate that internal quality systems are assessed independently and externally.**
- **supplement internal analytical controls via performance monitoring against other laboratories.**
- **provide an objective assessment of the reliability of the laboratory's methods and the validity of its results.**
- **compare the performance of different methods used by participants for a particular analysis.**

FAPAS® offers about 20 to 25 rounds for mycotoxins in different commodities per year. The results are confidentially evaluated and reported to the labs by FAPAS®.

Our laboratory participates in both, inter-laboratory comparison studies and the FAPAS® program.

In spring 2002 an inter-laboratory study was organised by the "Hungarian National Institute for Agricultural Quality Control" in Budapest. 4 different samples were sent to each laboratory, one uncontaminated sample and three samples contaminated with all mycotoxins that should be analysed. The laboratories were not informed about the fact that two of the three samples were identical. Analytical methods were not standardized across laboratories taking part in the study. Each laboratory was asked to apply its routine method. Half of the 50 participating labs returned the results.

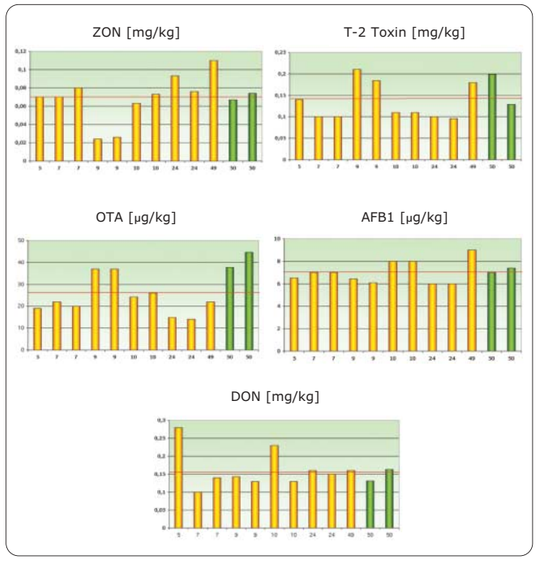


*Fig. 3: Inter-laboratory study, 2002*

60% of them used ELISA (Enzyme Linked Immuno Sorbent Assay), 30% HPLC (High performance liquid chromatography). The remaining 10% did not publish their method or used fluorometry or thin layer chromatography.

Figure 3 shows the HPLC results only. The average of all results is represented by a red line in the graphs. Our laboratory was given the number 50 (respective results are marked green).

Participations in FAPAS® rounds are already routine in our lab. They help us to constantly improve our methods and to assure the best service for our costumers. The following graphs show that we are definitely on the right way [figure 4]. The results of our lab are marked green. The red line indicates the respective toxin concentration of the sample.

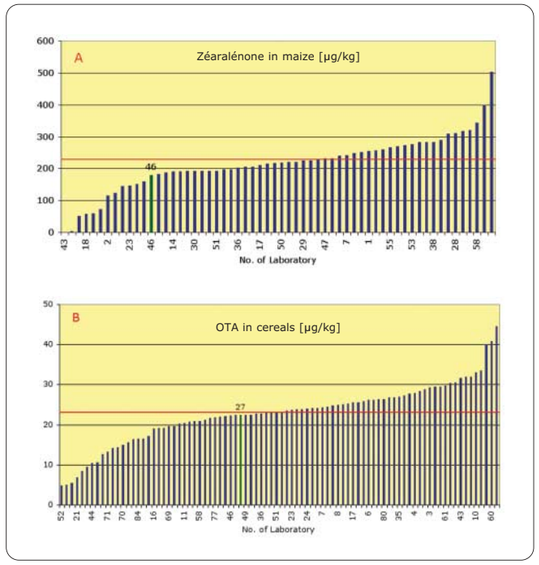


*Fig. 4: FAPAS®: Zearalenone [228 µg/kg] in maize (A) and OTA [23.39 µg/kg] in cereals (B).*

## Conclusions

**Many efforts have to be taken to guarantee constant quality of an analytical lab. Most of these procedures are rarely known by the costumer but nevertheless the validation process, the participation in inter-laboratory comparison studies and proficiency testing systems make the difference between labs and the difference between just a result or a reliable result.**