BioCop Woman in Science Fellowship Report

Hester van den Top

University:

UNIVERSITÄT ZÜRICH, Institut für Veterinärpharmakologie und –toxikologie, Switzerland
Director: Prof. F.R. Althaus
Host scientists: Hanspeter Nägeli, Katarina Lancova and Mirela Vitanescu.

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Initial aims

1. The overall aim is the transfer of the transcriptomics analysis method for the determination of trichothecenes to a chemical laboratory and to understand the different possibilities and limitations that analytical chemists encounter when applying transcriptomics.
2. The work plan is divided into two parts over the two-week period. During week 1 Hester will learn the cell culture techniques, RNA extraction, cDNA synthesis and quantitative RT-PCR analysis. While the second week will be spent in the home laboratory where the technique will be implemented with the help of staff from the UNIVERSITÄTof ZÜRICH.
3. The expected outcomes/benefits should result in a more fluid analysis procedure with a detailed description from sample extraction to transcript analysis. This is to be carried out at an analytical-chemical laboratory facility, such as the laboratory for Food and Residue Analysis at RIVM.

Technologies in which training was requested:

Cell culture techniques, RNA extraction and the RT-PCR / transcriptomics array tube technique.

Determination of trichothecenes in breakfast cereals.

The need for cheap and rapid methods to screen a wide range of both raw and manufactured foods for the presence of trichothecenes is (considering the various public health authorities that propose legislation to set strict allowable levels for type A trichothecenes in food) a subject that is of great interest. Present chemical methods use expensive equipment and give more quantitative information than might be needed for a screening method.

The introduction of transcriptomics based bioassays for the detection of type A and B trichothecenes for a chemist is something new.

The objective for my visit to the University of Zürich was to learn the new technique of using transcriptomics array for the determination of trichothecenes, and subsequently to transfer the method to the chemical laboratory (ARO) and to understand the possibilities and limitations analytical chemistry laboratories encounter when transcriptomics based methods are made operational.

My visit to the University of Zürich (TierSpital) has given me the opportunity to meet a dedicated and very enthusiastic group of people who were more than willing to demonstrating, teaching, and discussing all aspects of the transcriptomics analysis. During the two weeks training, two other scientists had also had the opportunity to learn more about transcriptomics analysis.

The first day of my visit, we learned about culturing cells specifically used for this transciptomic procedure. The MCF7 cells are easy to culture and we were shown the different stages of growth. Before exposing cells to the extracts that (may) contain trichothecenes the cells were starved using a

stripped medium that is estrogens-free. Not only had the medium to be estrogens free, but all plastic used in this phase of the procedure would also be free of estrogens.
After exposure of the cells to (breakfast-cereal) extracts, the procedure to harvest the cells was practiced and RNA was extracted from the cells. The RNA was transcribed to cDNA for performing one of the two techniques shown, the Real-Time PCR analysis for two identified gene expressions. All steps in this procedure were shown and explained in great detail as well as practical skills training.
I have acquired skills with working with RNA, cDNA and the precautions to be taken in the laboratory to prevent contamination at various stages of the procedures. Also the behaviour of RNA in a solution and the precautions to be taken to make sure the RNA will not deteriorate or be "lost" was emphasized.

The new transcriptomics method using array tubes with chips was then started. The cells that had been exposed to food extract containing trichothecenes were harvested using a scraper to detach the cells from the bottom of the wells. RNA was extracted using a shredder kit, isolated and transcribed to cDNA. The amount of MCF7 cells in each well in the 6 well plates should be the same, therefore when starting with this method, counting cells in the individual wells should be adopted as standard procedure. This practice should continue until a routine procedure is established to ensure that the amount of cells in each well is the same. At this time an experiment is started to test a 24 well plate for exposing the cells to food extracts. This will allow for a larger number of samples to be tested in one series.

The array tubes (provided by Clondiag – partner in the BioCop project) were prepared. This procedure was quick and easy to be adopted by a chemist. Some of the critical steps included the removing of the Triton containing buffer in the washing step, and taking precautions to ensure that the tube wall was free from any substance that may interfere with the next solvent to be applied. Pictures were taken with the Clondiag ATR 03 reader to detect the differences in the specific detectors printed on the chip on the bottom of the array tube, the software (in development) for transferring these different intensities of the spots was further applied. For me, the amount of information that is generated from these chips is enormous. The training provided details on how to interpret these readings, the different detectors on the chip for the trichothecenes, was both extensive and thorough.

After this week of training, it is my impression that this method is a very interesting technique and is a different method to quantify mycotoxins. One of the advantages (amongst others) of this technique is that it is an effect-driven quantitative assay.

I think that there are some points that may need to be addressed. The chip microarray itself is quick and easy, but culturing cells for this assay and preparing cells to be exposed to trichothecenes containing food extracts, takes longer and requires highly skilled technicians and special lab environment and equipment. The procedure from receiving samples (cereal based) to obtaining a quantitative result for trichothecenes T2/HT2 will take at least 3 days. At present the performance of this technique regarding e.g. precision in comparison with existing methods is not fully evaluated.

I would like to thank the Universität Zurich, Hanspeter Nägeli and especially Katerina Lancova for her patience in detailing instructions on all aspects of this technique. I also wish to thank Mirela Vitanescu for introducing me to the world of cell cultures. Finally my thanks also extend to Dr Chen Situ, the Gender Manage of BioCop project for her role in the BioCop Woman in Science Fellowship programme and the assistance she provided to carry out this visit.